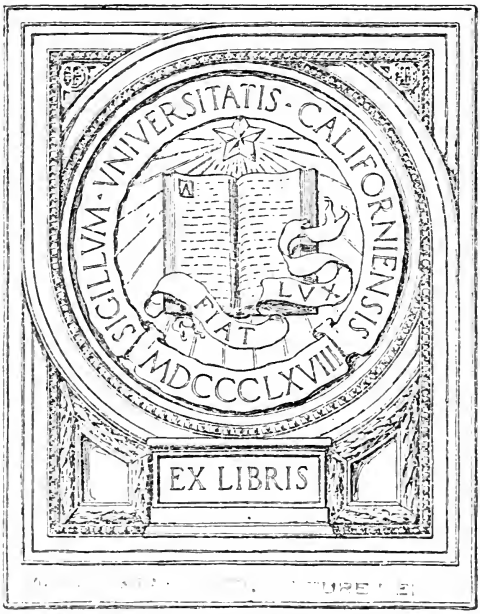
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VACCINE AND SERUM THERAPY IN VETERINARY PRACTICE

VACCINE AND SERUM THERAPY

IN VETERINARY PRACTICE

BY

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PREFACE

In writing this small volume I have endeavoured to explain the phenomena of immunity and make the explanation as clear of conception as possible. My chief object in producing this work is that it may be a small means of stimulating the veterinary practitioners of this country to take greater advantage of the modern means of treating disease by the various biological products now at their disposal. In this branch of medicine we hold a distinct advantage over the medical profession, inasmuch as we have a greater freedom in applying biologics in the treatment and diagnosis of the various diseases to which the domesticated animals are subject; also in serum treatment we have the advantage that the serum employed is very often obtained from the same species of animal as the one on which we are employing it. In other words, the serum is homologous. A great drawback in serum treatment of human patients is the fact that the immune bodies, or amboceptors, contained in immune serum obtained from the lower animals does not readily combine with the complement present in human serum, hence the maximum benefit

cannot be obtained from the use of such a serum. In veterinary practice this disadvantage need not arise. In dealing with the serum and vaccine treatment of the various diseases, I have only described the methods which come within the limits of practical application and from which best results are obtained. I trust this small work may be of some use to the veterinary practitioner in his daily conflict with the diseases of our domestic animals, and that it may be the means of stimulating a wider and more extensive application of modern biologics in veterinary medicine than at present exists in the British Isles. Should it do so, my object in its production will be attained.

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Barrow Elm House,
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July 21, 1922.

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VACCINE AND SERUM THERAPY IN VETERINARY PRACTICE

CHAPTER I

IMMUNITY: ITS CAUSE AND ITS EFFECT ON DISEASE

In contact with the bodies of animals there is a large flora of micro-organisms, some as constant parasites, others as transient invaders; some harmless saprophytes, and others capable of becoming pathogenic. It is evident, therefore, that the production of infection must depend upon other influences than the mere presence of the micro-organisms and their contact with the body, and that the occurrence of the reaction —for the phenomena of infection are in truth reactions between the germ and the body defences— is governed by a number of important secondary factors.

In order to cause infection it is necessary that the bacteria shall gain entrance to the body by a path adapted to their own respective cultural requirements, and shall be permitted to proliferate after gaining a foothold. Some of the bacteria then cause disease by rapid multiplication, progressively invading more and

more extensive areas of the animal tissues, while others may remain localised at the point of invasion and exert their harmful action chiefly by local growth and the elaboration of specific poisons.

The inciting or inhibiting factors which permit or prohibit an infection are dependent in part upon the nature of the invading germ and in part upon the defensive mechanism of the subject attacked. Bacteria are roughly divided into two classes—saprophytes and parasites. The saprophytes are those bacteria which thrive best on dead organic matter, and fulfil the enormous important function in nature of reducing by their physiological activities the excreta and dead bodies of more highly organised forms into those simple chemical substances which may again be utilised by the plants in their constructive processes. Parasites, on the other hand, find the most favourable conditions for their development upon the living bodies of higher forms.

While a strict separation of the two divisions cannot be made, numerous species forming transitions between the two, it may be said that the latter class comprise most of the so-called pathogenic bacteria. Strict saprophytes may cause disease, but only in cases where other factors have brought about the death of some part of the tissues, and the bacteria invade the necrotic areas and break down the proteids into poisonous chemical substances such as ptomains, or, through their own destruction, give rise to the liberation of the toxic constituents of their bodies. It is

necessary, therefore, that bacteria, in order to incite disease, should belong strictly or facultatively to the class known as parasitic. It must not be forgotten, however, that the terms are relative, and that bacteria ordinarily saprophytic may develop parasitic and pathogenic powers when the resistant forces of the invaded subject are reduced to a minimum by chronic constitutional disease or other cause. Organisms that are parasitic, however, are not necessarily pathogenic, and there are certain more or less fundamental requirements which experience has taught us must be met by the organism in order that it may be infectious for any given animal; and by infectiousness is meant the ability of an organism to live and multiply in the animal fluids and tissues. Variations in virulence occur, not only among different species of pathogenic organisms, but among bacteria of the same species. It is necessary, therefore, in order that infection may occur, that the particular organism invaded shall possess sufficient virulence.

Whether or not infection occurs depends also upon the number of organisms that gain entrance to the animal tissues. Bacteria, therefore, must be in sufficient number to overcome local defences and to gain a definite foothold and carry on their life processes before they can give rise to infection.

The Path of Infection.—The portal by which bacteria gain entrance is of great importance in determining whether disease shall occur or not. Streptococci when swallowed may be entirely in-

nocuous, whereas, if gaining an entrance through the skin, they set up disease. Conversely, typhoid germs may be rubbed into the skin, and may only set up a slight local reaction, whereas, if swallowed by man, they give rise to a dangerous disease. Animals are protected against bacterial invasion in various ways. Externally the body is guarded by its covering of skin and mucous membrane. When these are healthy and undisturbed, micro-organisms are usually held at bay, with exception, perhaps, of a few organisms such as tubercle bacilli, which may pass through the intestinal mucosa into the lymphatics, without causing local lesions. Even when bacteria gain entrance to the body by their most favoured channel, they are not always capable of producing disease. The bodies of animals have at their disposal certain general systematic weapons of defence, both in the blood serum and the cellular elements of blood and tissues, which, if normally vigorous and active, will usually overcome a certain number of the invading germs. If these defences are depressed, or the organism highly virulent, infection will take place. The disease processes arising as the result of bacterial invasion may depend wholly or in part upon the mechanical injury produced by the process of inflammation, the disturbance of function caused by the presence of the bacteria in the capillaries and tissue spaces, and the absorption of the necrotic products, resulting from the reaction between the body cells and the micro-organisms. To a large extent, however, infectious diseases are

characterised by the symptoms resulting from the absorption or diffusion of the poison produced by the bacteria themselves.

Bacterial Poisons.—The bacterial poisons are specific products of the bacteria, dependent upon the nature of the culture medium only as it favours or retards to the full development of the physiological functions of the germs. The poisons produced by all pathological organisms may be, to a greater or less extent, of several kinds. The true toxins are soluble, truly secretory products of the bacterial cell, passing from them into the culture media during their life. Such are the poisons of the tetanus and diphtheria bacilli. There are other micro-organisms in which no exotoxins are formed; to this class belong the greater number of pathogenic germs. These organisms during life do not produce true toxins; it is only after death and disintegration that the poison is liberated. This class of toxins is known as endotoxins. Therefore we have two classes of poison produced by micro-organisms: the true toxins, or extracellular poisons, and the endotoxins, or intracellular poisons.

The Mode of Action of Bacterial Poisons.—Study of the toxic products of various micro-organisms has shown that many of the bacterial poisons possess a more or less definite selective action upon special cells and tissues. Thus, certain soluble toxins of the tetanus bacillus and the *Bacillus botulinus* attack specifically the nervous system. Again, certain poisons produced by the staphylococci, the streptococci

and other germs, the so-called "hemolysins," attack primarily the red blood corpuscles. Others, again, act upon the white blood cells. We have seen that the mere entrance of pathogenic organisms into the bodies of animals does not always lead to infection. It is plain, therefore, that the animal body has some subtle means of defence, by virtue of which pathogenic germs are, even after their entrance into the tissues and fluids, disposed of, or at least prevented from proliferating and elaborating their poisons. The power which enables the animal to accomplish this is spoken of as resistance. When this resistance, which in a degree is common to all animals, is specially marked, it is spoken of as "immunity." Absolute immunity is exceedingly rare. The entire insusceptibility of cold-blooded animals under normal conditions with even the largest doses of many of the bacteria pathogenic to the warm-blooded animals is an instance.

The power of resisting any specific infection may be the natural heritage of a race or species, and is thus spoken of as "natural immunity." It may, on the other hand, be acquired, either accidentally or artificially, by a member of an ordinarily susceptible species, and is then called, "acquired immunity."

Natural Immunity.—It is well known that many of the diseases occurring in the human species do not infect the lower animals. Conversely, there are diseases of the lower animals which cannot be transmitted to man. Among the lower animals great

difference in the susceptibility and resistance to the various infections exist. Thus carnivorous animals are highly resistant to glanders and anthrax, whereas herbivorous animals are highly susceptible. This resistance in carnivora may be explained by the nature of the diet, as the carnivorous animals may have gained a slow artificial immunity from eating flesh contaminated with the organisms of the disease to which they show such resistance.

Acquired Immunity.—It is a matter of common experience that many of the infectious diseases occur but once in the same individual. This is notably the case in most of the exanthemata; resistance acquired in this way is called "acquired immunity."

Active Immunity: *Active Artificial Immunity.*—The process of conferring protection by treatment with either an attenuated form or sublethal quantity of the infectious agent of a disease or its products is spoken of as "active immunisation." There are various methods by which this can be accomplished. The following are the commonest:

1. By the use of attenuated cultures.
2. By the use of sublethal doses of fully virulent bacteria.
3. By the use of dead bacteria.
4. By using bacterial products.

Examples of the above methods in practice are: (1) Immunisation against anthrax; (2) present-day method of vaccination against rabies; (3) vaccines used against strepto- and staphylococci infections;

(4) black-leg aggressin (used for immunising calves against black-leg).

Passive Immunity.—In active immunity we have seen that such a condition was acquired by the animal by virtue of its own physiological activities, and it is self-evident that a method of this kind can, in the treatment of disease, be employed prophylactically only against possible infection, or in localised acute infections, or in disease which has a long period of incubation. A more hopeful method of treating some diseases is by the use of the serum of animals actively hyperimmunised against the specific disease. Thus by the use of antitetanic serum we can protect animals against the disease. The animal thus protected obviously had taken no active part in its own defence, but was protected from the action of the poison by the substances transferred to it in the serum of the actively immunised animal. Such protection is therefore a purely passive phenomenon so far as the treated animal is concerned, and the process is for this reason spoken of as "passive immunisation."

It is mostly in those diseases whose causative agents produce true secretory poisons that the production of passive immunity can be used with advantage; this is well exemplified in diphtheria and tetanus, by the use of their respective antiserum. Micro-organisms, however, which exert their harmful action rather by the contents of the bacterial cells than by secreted soluble toxins, do not, so far as is known,

produce antitoxins in the sera of immunised animals. The substances they call forth in the process are directed against the invading organisms themselves, in that they possess the power of destroying or of causing the dissolution of the specific germs used in their production. The therapeutic use of such anti-bacterial sera has been disappointing, and their prophylatic and curative action has been almost invariably ineffectual, except when the sera could be brought into direct contact with the germs, as in epidemic meningitis, when injected directly into the spinal canal. It should also be effective in enzootic spinal paralysis of equines, when used in like manner ; but, so far as I know, an antiserum against this latter disease has not been prepared.

Antibodies and the Substances giving Rise to them.—In the foregoing section we have seen that the process of active immunisation so changes the animal body that it becomes highly resistant against an infection to which it had formerly, in many instances, been highly susceptible. This fact started investigations to ascertain what part of the animal body was responsible for these changes, the result being that it was definitely proved that the blood and blood serum were chiefly responsible. During active immunisation the chemical composition of the blood serum is very little altered. The first actual light was thrown upon the phenomena of immunity by the investigations of Nuttall, Buchner, and others, who not only demonstrated the power of

normal blood to destroy bacteria, but also showed that this property of blood serum became diminished with age and was destroyed completely by heating to 56° C.

The thermolabile substance of the blood serum possessing this power was called by Buchner "alexin"; later Ehrlich renamed this substance, "complement," by which name it is now most commonly known. In 1890 von Behring, and soon after Ehrlich, made the important discovery that specific antitoxins could be produced in the sera of living animals against the poisons of some of the higher plants, and Calmette about this time succeeded in producing antitoxins against the poison of snakes, as well as against scorpion poison. Wassermann produced an antitoxin against the poison of the *Bacillus pyocyaneus*. Thus a large number of poisons of animal, plant, and bacterial origin have been found capable of producing specific antibodies in the sera of animals into which they are injected. The formation of antitoxins directed against soluble poisons did not, however, explain the immunity acquired by animals against bacteria like *B. anthracis* and others which, unlike tetanus, etc., produced little or no soluble poison. Much light was shed upon this phase by the discovery of Pfeiffer, who showed that when cholera spirilla were injected into the peritoneal cavity of cholera-immune guinea-pigs, the micro-organisms rapidly swelled up, became granular, and often underwent complete solution. This same

phenomenon could also be observed if the bacteria were injected into normal guinea-pigs, with a sufficient quantity of cholera-immune serum. Other workers also showed that this phenomenon would also take place *in vitro*, as well as in the animal body. The constituents of the blood serum which gave rise to this destructive phenomenon are known as "bacteriolysins."

Soon after Pfeiffer's discovery Gruber and Durham brought to light another specific property of immune blood serum. These workers noticed that certain bacteria when brought into contact with the serum of an animal immunised against them were clumped together, deprived of their mobility, and firmly agglutinated. The substance which produced this phenomenon they called "agglutinin." Later, Kraus demonstrated the presence of yet another specific antibody in immune serum. He showed that precipitates were formed when filtrates of cholera and typhoid bacilli were mixed with their specific immune sera. These substances he called "precipitins."

The treatment of the animal body, therefore, with bacteria or their products gives rise to a variety of reactions which result in the presence of the "antibodies" above described. Antitoxins, as we have seen, can be produced by a variety of poisons, either of animal or plant origin. Extensive investigations have also shown, that, likewise, lysins, agglutinins, and precipitins may be produced by the use of a

large number of different substances, chief among which are the red blood cells.

Bordet and others have shown that the serum of animals repeatedly injected with defibrinated blood of another species exhibited the specific power of dissolving the red blood corpuscles of this species. This phenomenon is known as hemolysis. Likewise, by using spermatozoa in place of blood, a serum can be produced which will seriously injure these highly specialised cells. To this latter substance the name of "cytolysins" has been given. The formation of cytolysins is not, however, general for all tissues of the body. The stimulation of antibody formation in the sera of animals is a consequence, therefore, of the injection of a large variety of substances—some of them poisonous, some quite innocuous. The substances possessing this power have been named "antigens."

The Toxin: *Antitoxin Reaction.*—The problem which now faces us is the manner in which toxin is made innocuous by the action of antitoxin. Ehrlich endeavoured to explain this phenomenon by conceiving that the reaction of toxin and antitoxin was a direct union, analogous to the chemical neutralisation of an acid by a base. Soon, however, Calmette and Wassermann, by experiments, were able to contradict this idea. These workers showed that the toxin only became inactivated by the presence of the antitoxin, the toxin again becoming reactivated after the destruction of the antitoxin by heat.

Standardisation of Antitoxins.—Shortly after the therapeutic application of antitoxin, it became apparent that no two sera, though similarly produced, could have exactly the same protective value. It was necessary, therefore, to establish some standard by which the approximate strength of a given antitoxin could be estimated. Von Behring, and later Ehrlich, attempted to do this by determining the quantity of immune sera which was needed to protect a guinea-pig of known weight against a definite dose of a standard poison. They ascertained the quantity of a standard toxin bullion which was able to kill a guinea-pig of 250 grams in from four to five days, and called this the "toxin unit." They spoke of a toxin bullion which contained one hundred such toxin units in a cubic centimetre as a "normal toxin solution," and designated as "normal antitoxin" a serum capable of neutralising, cubic centimetre for cubic centimetre, the normal poison. A cubic centimetre of such an antitoxic serum was, therefore, sufficient to neutralise one hundred toxic units, and was spoken of as an "antitoxin unit"; in other words, a unit of antitoxic serum is that quantity of serum which is able to protect a guinea-pig of 250 grams weight against one hundred lethal doses of toxin.

Ehrlich's Side-Chain Theory.—The extensive researches of Ehrlich into the nature of toxin-antitoxin reaction led him to believe that the two bodies underwent chemical union, forming a neutral compound.

The strictly specific character of such reactions, furthermore—diphtheria antitoxin binding diphtheria toxin, tetanus antitoxin only tetanus toxin, etc.—led him to assume that the chemical affinity between each antibody and its respective antigen depended upon definite atom groups contained in each. Ehrlich discusses the manner of cell nutrition, and advances the opinion that in order to nourish a cell the nutritive substance must enter directly into chemical combination with some elements of the cell protoplasm. The great number and variety of chemical substances which act as nutriment led him to believe that the highly complex protoplasmic molecules of cells were made up of central atom groups upon which depended the specialised activities of the cells and a multiplicity of side-chains, by means of which the cell entered into chemical relation with food and other substances brought to it by the circulation.

Just as nutritious substances are thus brought into workable relation with the cell by means of the atom groups corresponding to side-chains, so Ehrlich believes that toxins exert their deleterious effects only because the cell possesses side-chains by means of which the toxins can be chemically bound. These side-chains he calls "receptors." These receptors present in the cell and possessing by chance specific affinity for a given toxin are, by their union with toxin, rendered useless for their normal physiological function. By the normal reparative mechanism of

the body these receptors are probably cast off and regenerated.

Regenerative processes of the body, however, do not, as a rule, stop short at simple replacement of the lost elements, but usually tend to over-compensate. The receptors eliminated by toxin absorption are not, therefore, simply replaced in the same quantity in which they were lost, but are reproduced in excess of the simple physiological need of the cell. Continuous and increased doses of the poison, consequently, soon led to such excessive production of the particular receptive atom groups that the cell involved in the process soon becomes overstocked and casts them off to circulate freely in the blood. These freely circulating receptors represent the antitoxins. These, by uniting with the poison before it can reach the sensitive cell, prevent its deleterious action.

In the immediately preceding paragraphs we have dealt solely with immunity as it occurs where soluble toxins play an important part, and in which antitoxins are developed in the immunised subject. However, there are many species of pathogenic bacteria which stimulate little or no antitoxic substance when introduced into animals, and the resistance of the immunised animal cannot, therefore, be explained by the presence of antitoxin in the blood.

As mentioned, normal blood serum has the power of killing certain pathogenic bacteria, and the substance which gives serum this bactericidal power can be destroyed by heating the serum to a temperature

of 56° C. By adding to this inactivated serum normal fresh serum, it again becomes reactivated. From this observation Bordet drew the conclusion that the bactericidal action of the serum depended on two distinct substances. The one present in normal serum and thermolabile he conceived to be identical with Buchner's alexin; the other, more stable, produced, or at least increased, in the serum by the process of immunisation, he called the "sensitising substance." This substance, he believed, acting upon the bacterial cells, rendered them vulnerable to the action of the alexin. Without the previous preparatory action of the "sensitising substance" the alexin was unable to act, and likewise without the co-operation of the alexin the "sensitising substance" produced no visible effects. The same laws govern the phenomenon known as hemolysis as that of bacteriolysis.

Since the thermolabile substance or alexin or complement—more widely known by the last name—was always present in normal serum and had been shown to be little, if at all, increased during immunisation, this substance could have but little relation to the changes taking place in the animal body as immunity was acquired. The more stable "immune body" was the one which seemed specifically called forth by the process of active immunisation. According to Ehrlich's theory, then, it amounts to this, that when bacteria or blood cells are injected into the body of an animal certain atom groups or chemical components of the injected substance were united to

other atom groups or "side-chains" of the protoplasm of the tissue cell. These "side-chains" or receptors, then, reproduced in excess and finally thrown free into the circulation, constituted the immune body.

Ehrlich concludes that in the case of hemolysis complement does not combine directly with the corpuscles, but does so through the intervention of the immune body. This immune body, he reasons, possessed two distinct atom groups or haptophores: one, the cytophile haptophore group, with strong affinity for red blood cells; the other, or complementophile haptophore group, with weaker affinity for the complement. Because of this double combining power he speaks of the immune body as "amboceptor."

Agglutinins.—As has already been mentioned, immune serum has the property of agglutinating bacteria, and as this property is specific for the bacteria which produced the immunity, especially when an emulsion of high dilution of the bacteria is made, this fact is taken advantage of for bacterial species differentiation in clinical diagnosis. Widal's test for typhoid is an application of this agglutination phenomenon; and this test is of great importance, as the serum of patients suffering from this disease shows agglutinating power over the typhoid bacillus at early stages in the course of enteric fever.

Production of Agglutinins.—Just as normal serum contains small quantities of bactericidal substances, so does it contain agglutinins in small

amount. In a general way these "normal agglutinins" have the same nature as the immune agglutinins, and their presence is probably due to the presence of various micro-organisms parasitic on animals. Agglutinins may be produced in the sera of animals by the introduction of bacteria, subcutaneously and intravenously. As a rule they appear in the blood three to six days after the introduction of the bacteria.

Precipitins.—The precipitins, like the agglutinins, may be inactivated by heating to from 60° to 70° C., and cannot be reactivated by the addition of normal serum or by any other known method.

Specificity. — The specificity of precipitins is a question of very great importance, since these bodies have been used extensively for the differentiation of animal proteids. This specificity of precipitins has been used for forensic purposes by Wassermann, Uhlenhuth, and others, to distinguish the blood of one species of animal from another. Thus, blood-stains, dissolved out in normal saline solution, could be recognised by this reaction as originating from man or from an animal, even after months of drying. Precipitins have not been so far found in normal sera.

Multiplicity of Amboceptors.—As has been already shown, normal sera possess moderate bacteriolytic power, which can be destroyed by heating. Since such inactivated normal serum can be reactivated by the addition of fresh serum, it is plain that

the bactericidal power of normal serum, like immune serum, must depend upon the presence of amboceptor and complement. But normal serum often exerts lytic powers upon several species of bacteria, or, in the hemolytic tests, upon the red blood cells of several species of animals. It is supposed that this multiplicity of action is due to the presence in the normal serum of a variety of different amboceptors. The immunity acquired by an animal as a result of treatment with any of the various antigens is specific—for instance, an animal immunised against anthrax possesses marked bactericidal powers against the anthrax bacillus only.

The essential fact to remember is that the amboceptor alone enters into direct relation with the substance used for immunisation, and the specificity of immune sera therefore depends entirely upon the increase of amboceptor or immune body, and there is no corresponding increase of the complement; so the chief difference in a normal and immune serum, therefore, is the enormous increase of the specific amboceptors in the latter.

It might seem, from a consideration of the foregoing facts, that the explanation of immunity was very simple, but other ascertained facts render the question more complicated. Thus, taking the case of anthrax bacilli, we find that the blood of the rabbit, a highly susceptible animal, acts destructively upon the organisms in a test-tube; within the body it evidently does not do so. On the other hand, the

serum of an animal immune to the disease, as that of the hen, forms a good culture medium for the bacillus. The explanation of the phenomenon of the first example I shall deal with later, or, to be more precise, a possible explanation. The explanation of the later example is probably as follows: It has been shown by Browning, Zinsser, and Johnson, that the sera of animals, on standing, form an anti-complementary body, which destroys the function of the complement. Now, in the case of the hen's serum, *in vivo* it has a complete immunity to anthrax bacilli, but *in vitro*, on standing, it forms anti-complement, thereby losing its immune powers and so becoming a suitable culture medium for the bacilli.

Anti-complement and Anti-amboceptors.—There are many agencies which seem to interfere with a hemolytic or bacteriolytic system of any antigen-antibody-alexin complex. The so-called anti-complementary substances are many, among those of most interest being lipoids and globulins of the serum. Noguchi has demonstrated a lipoidal substance in many normal sera which directly inhibits the action of complement and is thermostable. Hemolytic sera, having the power of destroying red blood cells, must necessarily prove, in the presence of sufficient complement, to be a powerful poison when introduced into animals whose corpuscles they are able to injure. By careful and gradual dosage with such hemolytic sera, Ehrlich, Morgenroth, and Bordet have been able to produce immunity against

the hemolytic action; thus anti-hemolytic sera have been produced, the action of which may depend either upon the presence of anti-complement or anti-amboceptor.

The above authors, as well as Müller, have definitely demonstrated the presence of anti-amboceptor against hemolytic amboceptor. Here, then, we have two facts which may have enormous bearing upon bacterial infection, and on the course of the diseases which they produce. Let us take the case of normal rabbit serum and anthrax bacilli. It has been already stated that *in vitro* this rabbit serum is highly bactericidal for anthrax bacilli. In other words, the serum contains a large amount of immune bodies. The amboceptors being activated by the complement, rapidly destroy the bacteria. Now, *in vivo* what possibly happens is this. On the entrance of the bacilli an excess of immune body may be rapidly formed, and this excess, circulating freely, may become resorbed and give rise to anti-amboceptors, which at once inhibits the bactericidal or bacteriolytic action of the serum, thereby allowing the bacteria to rapidly multiply; or another phenomenon known as "deviation of the complement" may occur, caused by an excess of immune body in the serum, which also inhibits its bacteriolytic power; or the exact ratio of amboceptors and complement which gives the maximum degree of immunity may not be present. It is a fact that immune body and complement act in inverse ratio to each other; thus, if there is a deficiency of immune

body there must be an excess of complement, and *vice versa*, in the serum if its immunising power is to be effective.

If any or all of the above conditions occur in the serum of an animal, we have the explanation why a more or less mild and often chronic bacterial infection may suddenly become acute and highly virulent, thereby causing death. Similarly may also be explained the good results very often obtained by the old-fashioned method of bleeding as a therapeutic method of treating disease. By removing a certain quantity of blood, we also remove a certain quantity of immune body, thereby either preventing the formation of anti-amboceptors or the deviation of the complement. I will give some clinical cases to illustrate these points.

1. Cow ill with puerperal fever. This animal was treated medicinally, to which there was no response, and the cow died within forty-eight hours of my first seeing her. The owner of this cow had several others on the point of calving. Against my advice he allowed four more to calve in the same house in which the previous one died. These four developed the disease in a virulent form. One of these I treated medicinally, as in the former case, and three I treated in the following manner. From the jugular vein of each cow I removed 2 quarts of blood. Immediately afterwards I removed 6 pints of blood from each of four healthy cows. This blood was received into a glass jar containing some normal saline in

which was dissolved sufficient sodium citrate to make a ·5 per cent. solution when the blood was added. This prevented the blood from clotting. By means of a glass funnel, rubber tubing, and trocar and cannula, this citrated blood was slowly injected into the jugular of the diseased cow. The two other cows were treated in a similar manner. Before leaving the premises, I collected from healthy animals 6 quarts of blood. This was allowed to stand in a large glass jar for twenty-four hours, when the serum was decanted off. The amount of serum yielded from 6 quarts of blood varies from 32 to 36 ounces. Twenty-four hours after first bleeding and injection of citrated blood I again bled the animals, this time removing from each 1 quart of blood, and immediately after injecting into each 12 ounces of the serum obtained from the blood of the healthy animals. I repeated injections of normal serum, collected as above, for two more days, when the disease had declined and the animals were well on the way to recovery. The cow treated medicinally died fifty hours after my first seeing her. The three treated by bleeding and administration of normal serum made a complete recovery.

2. Cow suffering from severe sapremia, due to lacerations of cervix and floor of vagina, which occurred at time of parturition. Temperature on first examination was 105° F. Adopted treatment same as in No. 1, which was continued for three days, by which time the temperature had fallen to

101° F., when good nursing and tonics were continued. The animal made a rapid and complete recovery.

3. Half-breed mare suffering from septic intoxication due to retained placenta in non-pregnant uterine horn. The above treatment was adopted and continued for three days. In conjunction with this, uterine douches of ½ per cent. Lugol's solution of iodine were used. At the beginning of treatment the temperature was 106° F. On the fourth day the temperature was normal, and the animal made a complete recovery.

4. A gelding suffering from general septic poisoning, evidenced by multiple abscesses all over the body. The primary lesions were on the near hind-leg, and infection seemed to be carried by the lymphatics, as there was great thickening of the affected leg. Before applying the above treatment, I took swabs of pus for the purpose of having an autogenous vaccine prepared. However, as the animal was in a most critical condition, I decided to try bleeding and injection of normal serum. The method employed was as in No. 1, except blood was obtained from other horses. By the time I received vaccine, four days from date of sending swabs, the horse was well on the way to recovery, and the vaccine was never used. Although this animal was in an extremely emaciated and weak condition, he made a splendid recovery. I have also tried this treatment on several cases of pneumonia in horses, with the same excellent results. I attribute the good results obtained to the removal of excess of

antibodies and endotoxins by way of blood removed, and by the addition of fresh complement in the normal serum injected. This fresh complement activated the amboceptors already in animals' tissues to increased bacteriolytic action, at the same time stimulating the leucocytes to increased phagocytosis, with the consequent increased destruction and removal of bacteria and the neutralisation of their endotoxins.

Fixation of the Complement.—Bordet and Gengou devised a method whereby very small quantities of immune body can be demonstrated in a serum; their method is generally known as fixation of the complement. The following steps of the experiment explain this method:

A.	B.
Bacteriolytic amboceptor (plague-immune serum).	Normal serum heated.
+	+
Plague emulsion.	Plague emulsion.
+	+
Complement (fresh normal serum).	Complement (fresh normal serum).

To both of these, after five hours, was added—

Hemolytic amboceptor
(heated hemolytic serum).
+
Red blood cells.

Results:

(A) Showed no hemolysis. (B) Showed positive hemolysis.

In (A) the complement introduced in normal fresh serum has combined with the bacteriolytic amboceptor

contained in plague-immune serum—that is, it has become fixed, so that when later the hemolytic amboceptor contained in heated hemolytic serum is added there is no free complement present to activate it, therefore no hemolysis of the red cells occurs.

In (B) there is no bacteriolytic amboceptor present, therefore such complement remains free, to later combine with added hemolytic amboceptor, which it activates with a consequent hemolysis of red cells.

The Specificity of Hemolysins.—In the preceding paragraphs it has been shown that the blood cells of one animal injected into an animal of another species give rise to hemolytic substances in the blood serum of the second animal, which is strictly specific for the variety of cells injected. These hemolysins are called "heterolysins." Such hemolysins can also be produced by injecting the blood cells of one species of animal into another—for instance, by injecting the red cells of a goat into another goat hemolytic substances are formed in the blood of the previous goat, which are strictly specific for the animal from whom the red cells were taken; these hemolysins are called "isolysins."

So far, the experimental production of autolysins—that is, of substances in the blood serum which will produce hemolysis of the individual's own corpuscles—has not been successful. On the other hand, autolysins have been discovered in the blood serum of animals suffering from paroxysmal hemoglobinuria. In these cases the sensitising substance, or amboceptor, appeared to be absorbed by the red blood cells

only at low temperatures—probably in the capillaries during exposure to the cold—and hemolysis subsequently resulted in the blood stream by the action of complement. In this way probably azoturia is caused; the tissues of the body, which have become over-rich in proteid material by high feeding and rest of the animal, may during work liberate a proteolytic toxin, formed by the breaking up of the protein elements. These toxins probably have a hemolytic action on the red cells of the body, the hemoglobin being excreted through the urine.

Tests for Differentiation of the Various Organisms causing Disease.

By reviewing the preceding sections we observe that various phenomena are established in the serum of an animal suffering, and which has suffered, from a disease. By a practical application of each phenomenon, we have various means for determining the nature of the micro-organisms which produce the disease.

Agglutination Test.—This is extensively used in human medicine in making an early diagnosis of typhoid fever, and in veterinary medicine in diagnosing animals infected with contagious abortion. If we know the dilution at which organisms are agglutinised by a serum containing the specific immune bodies for the particular bacteria, this test has a wide range of application.

Precipitin Test.—This is used chiefly in human

medicine and for the determination of proteid material for forensic purposes.

Bactericidal and Bacteriolytic Tests.—The bactericidal and bacteriolytic powers of an animal's serum may either be tested in the animal's body or in a test-tube. The *in vivo* test is known as Pfeiffer's phenomenon. This depends upon the fact that bacteria, when injected into the peritoneal cavity of a guinea-pig, together with a homologous immune serum, undergo dissolution. As practised, the test finds a double application. It may be done to determine the bacteriolytic power of a given serum against a known organism, or for the identification of a particular micro-organism by means of its susceptibility to lysis in a known immune serum.

Hemolytic Tests.—Determination of the hemolytic action of blood serum, bacterial filtrates, and of a variety of other substances, such as tissue extracts and animal and plant poisons, have frequently to be made.

Fixation of the Complement Test.—This test is extensively used both in bacteriological investigations and in practical diagnosis for the determination in serum of specific antibodies.

CHAPTER II

PHAGOCYTOSIS

The studies in immunity which have been outlined in the preceding chapter have dealt entirely with the phenomena occurring in the reaction between bacteria or bacterial products and the body fluids. These studies have formed the basis of a theoretical conception of immunity formulated chiefly by the German school of bacteriologists. Parallel with these developments, however, investigations on immunity have been carried on which have brought to light many important facts concerning the participation of the cells of the body in its resistance to infectious organisms. The phenomenon which Metchnikoff and his pupils at the Pasteur Institute have studied in great detail, and upon the occurrence of which they have based their conception of immunity, is known as "phagocytosis."

It is well known that among the lowest unicellular animals the nutritive process consists in the ingestion of minute particles of organic matter by the cell. If the ingested particle is of an inorganic nature and indigestible, it will again be extruded after a varying time. If, however, the ingested substance is of a nature which can be utilised in the nutrition of the

protozoon, it is rapidly surrounded by a small vacuole, within which it is gradually dissolved and becomes a part of the cellular protoplasm. It is only as we proceed to animals of a much higher organisation that the function of cell ingestion of crude food is entirely removed from the process of general nutrition. Nevertheless, in these animals also the actual ingestion of foreign particles occurs, but it is now limited entirely to a definite group of cells.

In the higher animals this function of phagocytosis is limited to the white blood cells of the circulation, or leucocytes, to certain large endothelial cells lining serous cavities and bloodvessels, and to cells of rather an obscure origin which contribute to the formation of giant cells within the tissues.

A convenient division of phagocytic cells is that into "wandering" and "fixed cells." The wandering cells are the polymorphonuclear leucocytes; the fixed cells are those lining serous cavities and the blood and lymph spaces. When a foreign substance enters the body, it has at first a repellent action on the leucocytes; later it attracts them in large quantities. Such attraction is called "positive chemotaxis." In the case of bacteria, chemotactic attraction or repulsion is intimately dependent upon the nature of the micro-organism, and very probably has a definite relationship to its virulence.

We have seen that the invasion of the animal body by a foreign substance is followed by the prompt response on the part of the phagocytic cells. In the

case of bacteria, it has been shown that the virulent living organisms can be taken up by the polymorphonuclear leucocytes. The phagocytosis is, therefore, not simply a removal of the dead bodies of bacteria previously killed by the body fluids, but represents an actual attack upon the living and fully virulent organisms. Some organisms, however, show much greater resistance to phagocytosis than others, while some bacteria, as the tubercle bacillus, after ingestion by the leucocytes, oppose great difficulties to intracellular digestion. Cells of animal origin, or the dead cells of the animal's own body, are ingested by the large mononuclear leucocytes. Phagocytosis is much more active in animals which have a high degree of immunity to certain micro-organisms than in those that are more susceptible.

Opsonins.—Wright, by a study of the relation of the blood serum to phagocytosis in a series of experiments, proved conclusively that the serum contained a substance which acts directly upon the bacteria and not upon the leucocytes. This serum component is bound by the bacteria and renders them subject to phagocytosis. Because of their action in preparing the bacteria for ingestion by the leucocytes he named those bodies "opsonins." The importance of these opsonic substances in immunity was shown by Wright in a series of experiments, in which he determined that in patients ill with staphylococcus or tubercle infections the phagocytic powers were relatively diminished towards those micro-organisms, but could be specific-

ally increased by active immunisation with dead bacteria or bacterial products.

According to the theory of Wright, opsonins are distinct bodies that could not be identified with either complement or antibodies present in the serum. Other workers maintain that the opsonins are nothing more than lytic amboceptors or complement contents of serum. They base this contention not only upon the thermolability of normal opsonins, but also upon the fact that opsonins may be removed from normal serum at the same time as complement by the method of complement fixation.

Opsonic Index Test.—This test is applied when we wish to determine to what extent phagocytosis is occurring within the animal body in resisting a bacterial infection. The factors necessary for the performance of an opsonic test are (1) the blood serum to be tested; (2) an even emulsion of bacteria; (3) leucocytes. Next, equal quantities of corpuscles, bacteria, and serum, which are sucked into a capillary pipette, are mixed thoroughly by the repeated drawing in and out of the pipette's contents upon a glass slide. The mixture is then drawn into the tube, the ends are sealed, and it is placed in an incubator for from fifteen to thirty minutes. A control with normal serum is treated in the same way. After incubation the end of the pipette is broken off, the contents again mixed, and then smears are made upon a glass slide. The smear is next stained by any of the bloodstains. The smear is then examined under a microscope, and

the number of bacteria contained in such leucocytes counted. The contents of about eighty to one hundred cells are usually counted, and an average is taken. The average number of bacteria in such leucocytes is spoken of as the "phagocytic index." The phagocytic index of the tested serum divided by that of the normal control serum gives the "opsonic index."

I mention this test in detail, as it is one which can come within the reach of all practitioners, as practically the only requirements for performing the test are a small incubator, a microscope, and some bacterial cultures.

In applying vaccines in the treatment of disease, the opsonic test affords us an extremely useful guide as to the time the vaccine injections should be given, and as to the progress that has been made by the employment of vaccine.

Immediately after the injection of a vaccine there is a brief period during which the opsonic power of the patient is depressed below its original state. This is called the negative phase. The length of time occupied by the negative phase depends both upon the condition of the patient and upon the size of the dose given. After this, there is a gradual rise in the opsonic power, at first rapid, later slower, until a maximum is reached after a varying number of days. This period of rise represents the positive phase. The second inoculation of vaccine should, therefore, be made when the opsonic power is beginning to sink after the highest point of the positive phase. Should

a second inoculation be given during the negative phase, grave harm may be done; even the life of the animal may be endangered.

We have seen that the immune bodies established in the serum of an animal are specific for the bacteria employed in the immunisation; it is, therefore, obvious that, to have good results in vaccine therapy, it is absolutely necessary to employ a vaccine made from the specific organism causing the disease. This may not only apply to species, but to individual organisms of the species.

The proper course, therefore, to pursue is to employ an autogenous vaccine, but in some diseases—for instance, those caused by the streptococcus and staphylococcus—we may have a mixed infection of the cocci bacilli, some of which may be practically avirulent. In order, therefore to produce a suitable vaccine to combat the disease, we must determine which of the organisms found in the lesions are the highly virulent ones, and to prepare the vaccine from those. In infections where there is a mixed variety of bacteria, and we are not certain which type is causing the disease, the proper policy to pursue is to make a vaccine of each type, or, in other words, a mixed vaccine. The use of commercial vaccines in the treatment of diseases is in most cases useless, as they seldom or never contain the specific organism producing the disease.

Leucocyte Extract.—In the preceding paragraphs upon opsonins and phagocytosis is discussed the

protective action exerted by the living leucocytes against bacterial infection, and the relation of these cells to the blood serum; furthermore, that while our knowledge of the blood serum, as developed at present, shows that phagocytes may be aided by this in the ingestion of bacteria, the subsequent digestion of the germs, and possibly the neutralisation of their intracellular poisons, is, as far as we know, largely accomplished by the unaided phagocytic cell. The thought is obvious, therefore, that in the struggle with bacterial invasion the leucocytic defenders might be greatly reinforced if they were furnished as directly as possible with a further supply of the very weapons they were using in the fight with the microorganisms. Hiss has produced an extract of the chief cells usually found in exudates, as he assumes that extracts would be more potent than the living leucocytes themselves, since, if diffusible, they would be impartially distributed to all parts of the body by the circulation. They would then, as quickly as absorption would permit, relieve the fatigued leucocyte, and also protect, by any toxin-neutralising or any other power they might possess, the cells of highly specialised functions. Hiss claims to have very good results in the treatment of staphylococci and streptococci infections by the use of this leucocytic extract.

Several other authors have shown that these leucocytic extracts contain bactericidal substances which are called "endolysins." These substances in structure are quite different from that of serum

bacteriolysins, nor are they increased by immunisation, the quantity present in each leucocyte being probably at all times simply sufficient for the digestion of the limited number of bacteria which can be taken up by the individual cell.

Problems and Facts of Immunity in their Bearing upon the Treatment of Infectious Diseases.—While dealing with the various theories and facts in the previous sections, no systematic attempt has been made to correlate the facts presented or to determine their bearing on the most vital problem of all, the treatment of infectious diseases. To understand this more fully, let me recall certain facts, some of which are well understood — for instance, the fact that certain micro-organisms, such as the tetanus bacillus, secrete soluble poisons, both during artificial cultivation and during their life in the animal body, which poisons are highly toxic. Such germs, then, once having gained a firm foothold, and often even an insecure foothold, in the animal body, are possessed of a powerful weapon of offence against the sensitive physiological bases of the host, and, possibly, of defence against its more immediate and mobile means of combating the germs themselves.

In the case, however, of most other pathogenic germs, the secretion, at least in artificial media, of such highly soluble poisons has not been satisfactorily demonstrated. In the case of the bacteria which secrete true soluble poisons, these poisons are

neutralised by their antisera, unit for unit, according to the law of multiples. Now, however, in the case of the vast majority of pathogenic bacteria, which belong to the class of micro-organisms which owe their toxicity to the endotoxins contained within their bodies, these latter poisons are said to be liberated from the dead organisms during their disintegration or during autolysis. These endotoxins are recognised by the fact that they do not call out true antitoxins which become free in the plasma and serum, but do, nevertheless, lead to the formation of digestive antibodies, these not, however, following the "law of multiples" in protecting infected animals from the poisons. The liberation of these poisons by the destruction of the bacteria in the animal body is well illustrated by the so-called phenomenon of Pfeiffer which takes place when cholera vibrios and immune cholera serum are introduced into the peritoneal cavity of a guinea-pig. If specimens are withdrawn from time to time, a rapid swelling up, disintegration, and disappearance of the vibrios can readily be demonstrated.

The power of disintegration is also claimed for the body fluids of normal animals, and is supposed to be demonstrated by the following experiment. When graded quantities of a fresh cholera culture are introduced into the peritoneal cavity of normal guinea-pigs of equal weight the following phonemena can be observed: Minimal doses of the culture produce a febrile condition which continues for a few hours with

no serious symptoms. Slightly larger doses give rise to definite symptoms of cholera poisoning. These symptoms of poisoning gradually disappear, and after twenty-four hours the guinea-pigs are again normal. If the quantity of cholera culture is carefully increased up to the minimal lethal dose, the animal dies with all the typical symptoms of cholera intoxication, but on autopsy the peritoneum is found to be perfectly sterile. Finally, if larger quantities of living cholera spirilla are injected, the peritoneal cavity shows a profuse, serous, and sometimes a hemorrhagic exudate, which contains innumerable active micro-organisms.

This experiment demonstrates the fact that the normal guinea-pigs which receive enough of the cholera vibrios to prove fatal have destroyed the vibrios and presumably died from the poisons thus liberated, and not from the poisons secreted by the living vibrios. It is only when the animal's system is previously flooded with an overwhelming dose that the vibrios are found alive and multiplying even locally in the peritoneum after death. This does not prove, however, that no multiplication goes on hand in hand with the destruction of germs in the infected animal; on the contrary, such a multiplication is rather the rule than the exception. This has been well illustrated by the experiments of Pfeiffer and Wassermann, who, after having shown that the blood serum of humans who have recovered from Asiatic cholera has the power to protect guinea-pigs from

ordinary fatal doses of the cholera spirilla, even when used in high dilutions, proved that this protective power is not an antitoxic one, but depends largely, if not entirely, on the ability of the serum to aid in the immediate dissolution of the vibrios. Now, however, if the dose was increased to a much larger amount, not even ten thousand times the original amount of serum would protect the animals against the inoculation.

Now, let us analyse the above facts and seek an explanation of their phenomena. In Pfeiffer and Wassermann's experiments we have seen that even a small amount of cholera-immune serum, when injected into a guinea-pig, protected that animal from a normally fatal dose of cholera vibrios. But, by the phenomenon of Pfeiffer, we have seen that the antibodies in such an immune serum are to all intents and purposes bactericidal—that is, they cause the dissolution and disintegration of the bacteria, but at the same time that the bacteria are undergoing disintegration their poisonous substances, the endotoxins, are liberated; and as it is these endotoxins which produce the toxic symptoms in the animal body, there must be some other factor than the antibodies within the animal tissues to overcome the amount of toxins which must be liberated from the injection of ordinarily fatal doses of cholera spirilla in the Pfeiffer and Wassermann experiment.

We have already seen that the neutralising substance for endotoxins is contained within the leuco-

cytes, therefore in the above experiment phagocytosis must be sufficiently active to engulf the disintegrating vibrios and at the same time neutralise their liberated endotoxins to such an extent that they had no visible reaction on the guinea-pig. The leucocytic activation must be due to what Wright calls opsonins, but what other authorities believe to be nothing more than the bacteriolytic or bactericidal amboceptors contained in the immune serum.

We next come to the latter part of the phenomenon occurring in the above experiment. On the guinea-pig recovering from the first injection of immune serum and ordinarily fatal dose of cholera vibrios, if it be given, say, five times the original dose of vibrios, not even ten thousand times the amount of the previous dose of immune serum will protect it. What is the explanation of this? Personally, I think it is the following: During the reaction between the tissue fluids, the immune serum, and injected vibrios, within the body of the guinea-pig, caused by the first injection of immune serum and virulent vibrios, there was an active production of immune bodies in the guinea-pig's serum. If these bodies were produced in excess, they were liable to become resorbed, thereby giving rise to anti-antibodies; therefore, on the animal recovering from the effects of the first injection of immune human serum plus an ordinarily fatal dose of vibrios, its serum contained free antibodies and anti-antibodies. When the guinea-pig receives the second, but larger, dose of vibrios, a new

stimulus is given to its already sensitised cells to produce a further supply of immune bodies; these, being much in excess of the normal physiological requirements, may become quickly resorbed, thereby giving rise to a further production of anti-anti-bodies. Now, if the anti-antibodies be in excess, their primary action is to alter the phagocytic powers of the leucocytes, by weakening or destroying their neutralising property, and by so doing enabling the engulfed vibrios to destroy the leucocyte, with its consequent disintegration and liberation of the contained toxin. Accordingly, as the amount of anti-antibodies increases, the bacteriolytic or bactericidal action of the serum decreases, the result being an enormous increase in the virulent living organisms within the animal body, which by this time has probably succumbed to the overwhelming amount of toxin liberated. We thus find that a guinea-pig which receives the minimum lethal dose of cholera vibrios dies with all the symptoms of cholera poisoning, death being caused by the endotoxins liberated from the vibrios, which have been engulfed by the leucocytes; for, on autopsy of the guinea-pig, there are no vibrios to be found. We therefore see that, although phagocytosis was sufficiently active to remove all the micro-organisms, yet the leucocytes had lost their power to neutralise the endotoxins of the bacteria they had engulfed. Again, if the amount of virulent organisms injected be increased above the minimal lethal dose, the animal dies with the same

symptoms as before, but on autopsy the peritoneal cavity shows a profuse serous exudate, which contains innumerable actively motile micro-organisms. In this case we find that not only has the serum lost its neutralising property, but it has also lost its bactericidal and phagocytic properties as well.

By formulating the above theory, I am perhaps laying myself open to severe criticism, but by a careful study of the facts revealed by Pfeiffer and Wasserman's experiments and the clinical observations I have previously mentioned, this seems the only possible explanation of the phenomena occurring within the body of an animal suffering from a virulent bacterial invasion.

Reviewing all the above facts and theories, let us see what relation they bear to the prevention and treatment of disease. Take first those diseases caused by bacteria which secrete true soluble toxins. It is by the action of these toxins upon the sensitive physiological cells of the animal that the disease manifests itself. In veterinary medicine the commonest example of this class of disease is tetanus. Tetanus-immune serum is able to neutralise tetanus toxin unit for unit, but this neutralisation must be accomplished before the toxic atom attaches itself to the cell receptors; therefore, it is necessary that antitetanic serum must have a chance of neutralising the toxins of tetanus bacilli while they are freely circulating in the tissues—in other words, the specific use of the antiserum is that of a preventative. At the

same time, it must be remembered that the immunity set up by the use of this class of serum is of the passive kind, and has but a limited duration, lasting on the average about eight to twelve days; it is therefore essential that, in order to keep an animal immune to this class of disease, it is necessary to give it an injection of the antiserum about every ten days.

Now, when we turn to those diseases caused by micro-organisms which do not secrete true soluble toxins, we cannot produce antisera for the bacteria responsible for such diseases, which will contain antitoxic substances; however, such sera will contain immune bodies, in the form of either bactericidal or bacteriolytic substances. Up to the present, nothing but disappointment has persistently followed in the wake of serum therapy as applied to these infectious diseases. This disappointment, I believe, is due to the formation of anti-antibodies which I have already mentioned.

To give animals immunity against diseases caused by this latter class of micro-organisms, we must make use of vaccines. The administration of bacterial cultures for purposes of prophylaxis is a procedure which is easily explicable on ordinary principles, since the introduction of a dose of virus which the individual is capable of overcoming by means of his natural powers of resistance ensures a supply of antibodies being formed to resist subsequent attacks by the infective organisms, since to confer active

immunity it is necessary to introduce the specific virus into the animal to be immunised.

At the same time, it is evident that to introduce the infective material into an animal by the same channel as that by which infection enters in the naturally acquired disease would merely induce the very conditions from which it is sought to gain protection. Some other method must be selected. Several different ways of inoculation without conveying an actual attack of the disease are available:

1. By use of attenuated virus.
2. By using the dead bodies of the bacteria.
3. By inoculating the bacteria in some special way, different from that by which they normally enter the body to cause infection.
4. By the injection of an antitoxic serum at the same time as the injection of virulent organisms is made.
5. By inoculating a very small number of virulent germs. This is the method now used to protect against rabies.
6. By sensitising the bacteria before inoculation. This is accomplished by mixing the bacterial emulsion with its heated specific antisera and allowing the mixture to stand for twelve hours.
7. Prenatal immunisation. This is a method which has been very little used, if at all, in this country. It consists of immunising the fœtus, through the maternal blood—that is, the pregnant mother is inoculated with the specific virus of the

disease from which we wish to protect the offspring immediately after birth. This method is applicable to such diseases as "joint ill" and "white scour" in young animals. Where this method has been used, it has been found highly effective in protecting young animals against the two above-mentioned diseases.

In the treatment of bacterial diseases, vaccine therapy has been successful only in chronic and more or less localised conditions. In septicemia, the use of vaccines has not met with any beneficial results. In those conditions where the lesions are local, vaccine treatment is very often most successful. The explanation of this is, that in local infections the bacteria establish themselves at a particular spot in the tissues which has little resistance—*i.e.*, little power of producing antibodies—and the injection of a vaccine in some healthy distant situation may stimulate the formation of such bodies elsewhere; they may then be brought to the infected area by the blood, provided there is a free supply of lymph transuding into the affected part.

In conclusion, I must point out that in order to treat a disease successfully with either a serum or vaccine, it is first necessary to correctly identify the organism causing the disease. I am afraid that in veterinary practice this fact has been sadly neglected, the results obtained from this method of treatment being often very poor and indifferent.

CHAPTER III

ANAPHYLAXIS

When the serum of one species of animal is injected into an individual of another species, antibodies are formed, as has already been described. But, in addition to this formation of antibodies, or as a part of the process, changes take place in the infected animal; by these it is rendered peculiarly sensitive to a further injection of the same kind of serum. Symptoms of severe constitutional disturbances, and often even death, may ensue upon a second injection. Thus, if an animal receives an intraperitoneal injection of horse serum it suffers no ill effects, but if the injection is repeated in about twelve days' time, it is liable to become seriously ill, with symptoms of collapse, bloody urine and fæces, dyspnœa, and convulsions, and may die rapidly or instantaneously. The phenomenon is known as anaphylaxis.

This condition is not confined to the use of serum, but is established when proteins of a wide range are employed.

This hypersensibility does not develop immediately, but only after the lapse of a varying interval, generally eight to ten days, varying with the protein used and

animal inoculated. It may pass off gradually in a month or more, or may persist for an indefinite period. A very minute quantity of protein at the initial injection is sufficient to sensitise the animal; it is necessary that the second injection be of much greater quantity to produce anaphylactic symptoms. The time at which the second injection gives rise to the most violent symptoms, moreover, is to a large extent dependent upon the size of the sensitising dose: the smaller the initial dose the shorter the incubative period of the anaphylactic state. At reinjection the symptoms are most severe when the injection is made intravenously.

In an animal suffering from anaphylactic shock there is lowering of the blood pressure and a decrease in its coagulability. When sensitised animals recover from the second injection they are thereafter immune —that is, they do not react to the subsequent injections of the same substance. This desensitisation, or anti-anaphylaxis, as Besredka has named it, appears immediately after the recovery from the second injection.

Anti-anaphylaxis may also be produced if animals which have received the first or sensitising dose are injected with comparatively large quantities of the same substance during the pre-anaphylactic period. This injection should not be given too soon after the first dose, but rather toward the middle or end of the pre-anaphylactic period.

Anaphylaxis is a strictly specific condition; so

much so, that it is now taken advantage of to determine different kinds of protein. Besredka claims that by the anaphylactic reaction he has been able to determine the human or animal nature of mummies many thousands of years old, and that in these cases the precipitin and fixation of complement tests proved quite ineffectual. It has been shown that this hypersensibility can be passively transferred to normal animals by injecting them with the serum of anaphylactic animals. This condition may also be transmitted by inheritance.

A number of theories of anaphylaxis have been advanced, but most authorities have now come to agree with the one propounded by Besredka of the Pasteur Institute. This theory is that anaphylaxis is the result of the presence of specific antibodies in the animal into which the antigen is injected. These antibodies are now believed to be attached to the body cells, and the anaphylactic shock to be produced when the antigen comes into contact with these antibodies.

The following is the text of the hypothesis of anaphylaxis put forward by Besredka. He refers to the antigen as sensibiligen and the antibody as sensibilisin. "What takes place at the time of the second injection? The newly arrived antigen comes into contact with the already formed sensibilisin. The effect of this affinity is to produce an intense reaction. Whether this reaction disturbs the equilibrium of certain nerve cells where the combination takes place, or whether

the latter is accompanied by the setting free or the absorption of energy, calorific or otherwise, we have presented to us a series of phenomena always the same, and which constitute the anaphylactic shock. What governs anaphylaxis and anti-anaphylaxis is neither the toxin nor antitoxin, but, on the one hand, the rate at which the sensibiligen and the sensibilisin come into contact; and, on the other, the place where they meet, which is probably the nervous system."

In serum treatment our object must be to prevent anaphylaxis in patients we consider may be susceptible to this condition. To do this we must establish a condition of anti-anaphylaxis. Several methods of producing anti-anaphylaxis have been employed, such as by anæsthetising the animal preparatory to injection of serum; by heating the serum to 60° C. on three or four successive days; and by injecting the patient with small graduated doses of serum. This last method is one now universally employed. When using this method we have to remember that the time taken for anti-anaphylaxis to be established depends upon the route by which the antigen enters the animal body. Thus, by the subcutaneous route it takes from three to six hours; by the intraperitoneal route one to two hours; and by the intravenous method ten to fifteen minutes. It is obvious, therefore, that the intravenous method of anti-anaphylactic vaccination is one to be desired. Let us remember that anti-anaphylaxis is nothing more than the desensitisation of the sensitised animal.

When, to obtain anti-anaphylactic immunity, we employ the method of repeated small doses, we are only provoking a series of slight successive anaphylactic shocks; the great shock is thus broken by the reaction being made slower and being divided into small doses. Anaphylactic shock is also a desensitisation, only, instead of being slow, it is rapid; therefore, the whole difference in anaphylactic shock and the changes that take place in the formation of anti-anaphylaxis is in the time of reaction.

Clinically, especially in canine practice, when we do not know the previous history of our patient, if we are about to employ serum treatment, we should always have resource primarily to anti-anaphylactic vaccination. For all we know, the patient may have had a previous dose of serum, and is, therefore, in the sensitised condition. Only a very short time ago this fact was brought very forcibly to my notice. In a fox-hound kennel I have seen three puppies die and four become violently ill from the use of antidistemper serum. The first dose of serum sensitised these puppies, and the second dose caused the anaphylactic shock. These puppies were attacked with anaphylactic symptoms within ten minutes of receipt of second injection.

The symptoms of anaphylaxis I have described are seen chiefly in dogs and other small animals. The symptoms in cattle and horses are swelling and œdema of the head, especially around the muzzle, excitement, colic, and bloody diarrhœa; in severe

cases paralysis of posterior extremities, which may end fatally or complete recovery may result within a few hours. Marked intolerance by some animals to certain drugs, which intolerance was described as being due to idiosyncrasy, has now been found to be drug anaphylaxis; this intolerance is often seen with iodoform, antipyrin, quinine, and the different bromides. The mallein and tuberculin reactions were thought to be due to anaphylaxis, but Besredka and others seem to have conclusively proved that such is not the case.

CHAPTER IV

DISEASES PRODUCED BY BACTERIA WHICH SECRETE TRUE TOXINS

Tetanus, or Lockjaw.—Causal organism—the *B. tetani*—was discovered by Nicolaier in 1884. The bacillus, in the form of spores, is widely distributed in nature, being commonly found in manure and soil which has been manured. It is also frequently found in the intestinal contents of horses and cattle. Entering the body by means of a wound in the skin or mucous membrane, the bacilli do not become generalised, but remain localised at the point of infection, where they produce their toxins. These are taken up by the peripheral nerves and are carried along the axis-cylinders to the central nervous system.

Meyer and Ransom, as the result of many experiments, consider that it is only by the peripheral nerves that the poison can reach the spinal cord or brain. Poison circulating in the blood is taken up by the nerve endings, and so passed on to the central portions of the nervous system, but it does not penetrate directly into the latter from the lymph.

They thus explain the incubation period met with in poisoning with tetanus toxins. They point out

that incubation is longer in direct proportion to the size of the animal and the consequent length of the nerves. In horses and cattle the symptoms are exhibited after an incubation period of five days; in smaller animals two to four days; but in all cases the incubation period may be very variable. The more rapid the onset, the more acute are the symptoms, and the more grave the prognosis.

The poison of tetanus gradually loses strength on keeping, and is not destroyed by heating to 135° C. for ten minutes; but its potency is rapidly destroyed by sunlight. The poison contains two toxins, tetanospasmin, which produces spasms of the muscles, and tetanolysin, which causes hemolysis of the red blood corpuscles. Tetanus toxins have a great affinity for the nervous system. Wassermann and Takaki have shown the great affinity of the poison for brain tissue; they found that if they made an emulsion of brain substance and mixed this with the toxins before injections into animals no ill-effects were produced. A protective influence was exercised even if the emulsion was injected at a different point from the toxin, without previous mixture. Cerebral substance thus acts as an antitoxin to the poison—in other words, the toxin has the power of combining with the side-chains of the cerebral cells. When, therefore, these cells are injected into another animal, they are capable of uniting with the free poison and so preventing it from attacking the living tissues of the animal.

Preparation of Antitoxins.—Horses are used for the practical preparation of the antitoxin. Great care must be exercised in the process of immunisation, as these animals are very sensitive to the toxins. At the beginning of the treatment use is made of a toxin attenuated by some chemical agent (iodine trichloride) or by mixture with antitoxin, which is injected subcutaneously or intramuscularly in increasing doses at intervals of from five to ten days. This treatment is continued until the animal is hyperimmunised or until sufficient antibodies are formed in the blood serum.

Standardisation of Antitoxins. — Behring's method, in which 1 unit of antitoxin will protect a guinea-pig against 1,000 minimal lethal doses of toxin.

Rosenau and Anderson take as a unit of antitoxin ten times the least quantity of antitoxin which will keep a guinea-pig (of 350 grams) alive for ninety-six hours after an injection of an official toxic unit. This latter unit is the equivalent of 100 minimal lethal doses. Unfortunately no antitoxic unit has gained universal acceptance in the description of the various sera on the market, the dose being generally calculated in cubic centimetres of serum, without any statement of the number of units contained. There is at the present time a great tendency to adopt the U.S.A. antitoxic unit, and MacConkey states that the German (Behring) unit is equivalent to about 40 U.S.A. units; 1,600 Italian units are equivalent to 1 U.S.A. unit; and the usual prophylactic dose of

10 c.c. of the Pasteur Institute serum is equal to about 600 U.S.A. units. By remembering the above figures and noting the make of the serum, we can estimate the number of units contained in a dose of each individual serum.

Experimental Value of Antitoxin.—From laboratory experiments, there can be no doubt that tetanus antitoxin, if given along with or shortly after a dose of toxin, has the power of preventing the characteristic symptoms and of death. Accordingly, as the time between the injection of toxin and antitoxin increases, so must the quantity of antitoxin increase if its beneficial effects are to occur. Kraus and Amiradziti further showed that the antitoxin does not penetrate the toxin-loaded cells, but that the toxin must diffuse out before it can be neutralised, the rate of diffusion being accelerated by the presence of a high concentration of antitoxin in the surrounding fluid. But there comes a time when the toxin has entered so closely into combination with the cells that no amount of antitoxin is capable of withdrawing it. After this time even the minimal lethal dose is of necessity fatal.

Further, even if the blood of an animal is rendered antitoxic to tetanin, injection of this poison into the substance of its brain will still produce the fatal effects.

Prevalence of Tetanus.—Tetanus is a common disease of the domesticated animals, especially among equines, and in most cases is fatal. Before any

objective symptoms of the disease are exhibited, a lethal quantity of the toxins have attached themselves to the cells of the nervous system, and in such a condition, it being impossible to overcome or neutralise these poisons, a high mortality from this disease is the result.

What are the phenomena which govern the special affinity of tetanus for the cells of the nervous system? Is it that this poison has some special constituent that has the power of linking it to the special nervous cells? or is it some constituent of these cells that has this affinity for the poison? The weight of evidence goes to pronounce this in the favour of the latter.

Uses of Antitoxin in Prevention and Treatment of Tetanus.—As a prophylactic antitoxin may be almost considered a specific. As the immunity conferred on an animal by the use of a dose of antitoxin is only passive, therefore in order to keep this immunity established it is necessary to give doses of antitoxin at intervals of at least every ten days, as it has been shown that this immunity cannot be relied on to extend beyond this period. As a prophylactic 3,000 U.S.A. units are sufficient for one dose. In cases that are not urgent this is generally given hypodermically. Before discussing the curative effects of antitoxin, it is best to describe the various modes of administration.

1. **Subcutaneous Method.**—This is the mode frequently employed when the antitoxin is given as a prophylactic; but as the antitoxin is only slowly

absorbed by this route, one or two days elapse before the maximum concentration of antitoxin in the blood is reached; whereas in treatment of the disease it is important to neutralise the toxin, which has already got the start of the antidote, as quickly as possible; hence intravenous injection is much to be preferred to the subcutaneous route, as being speedier in action —a question of minutes only.

2. **Intravenous Method.**—The jugular vein is the site most suitable for the injection. The most rigid aseptic precautions must be observed. The injections can be given either with a large hypodermic syringe or by any of the appliances used for intravenous injections. Both of these methods effect a neutralisation of the poison circulating in the blood stream, but they do not avail to counteract that which has already reached the nervous system. Two methods adopted to achieve this end, if it be possible in any way, have been recommended—namely, the injection of the antitoxin into the space between the dura mater and the brain (subdural) or spinal cord (intrathecal), and injection directly into the brain substance (intracerebral). For ease and safety of administration and generally for effectiveness, the injection of the serum intraspinally after lumbar puncture is undoubtedly the best. By making use of this last method, I have personally seen five recoveries out of six cases of tetanus in horses; but in conjunction with this treatment the animals were also kept in a condition of semihypnosis by the use of chloral hydrate. I shall

refer to this latter treatment later. Behring considers that no good results are to be hoped for from the use of antitoxin if it is administered more than thirty hours after the onset of symptoms, or if less than 100 units (on his system) are given. Even if these postulates are fulfilled, no great results can be looked for.

In treating an animal with tetanus, the first duty is to locate the point of infection if possible; having done this, next proceed to open up the part, so as to enable the removal of any pus or necrotic material. The parts should then be cauterised, after which antitoxin should be injected into the wound and into its immediate surrounding neighbourhood. Next intravenous injections of the antitoxin should be given: doses of 200 units of Behring's serum, 100 c.c. of Pasteur's serum, and 18,000 U.S.A. units should be given. These doses should be given at least twice daily, and if given three times daily better results can be hoped for. Next inject intrathecally 20 c.c. to 40 c.c. This is best done in either the lumbar region (lumbar puncture) or in the space between the occipito-atlantal articulation. The site for the former method is the space between the fourth and fifth lumbar vertebræ, or at a point where a straight line drawn from the most anterior part of the external angle of ilium on one side to the same point on the other side cuts the line of the lumbar vertebræ at right angles. For this injection a needle 10 to 15 cm. long is required or a small trocar and cannula may be used,

and should be made of some tough, unbreakable material. The animal should be placed in such a position that the fore and hind legs approximate, so that the back is arched; the needle is then pushed perpendicularly into the spinal canal at the site indicated. When the canal is reached, which is indicated by absence of resistance to the needle, there is an escape of intraspinal fluid. If this does not come of its own accord, as often it does not, a quantity up to 10 to 20 c.c. should be withdrawn; at the same time the animal's pulse should be carefully observed for any sudden alteration. On this appearing withdrawal of spinal fluid should be stopped. Having withdrawn the spinal fluid, the antitoxic serum is injected very slowly into the spinal canal. The intraspinal injection should be made once daily.

A method of treatment from which I have had good results is a combination of antitoxin, given by the different methods mentioned, and large doses of chloral hydrate. My procedure was to give $\frac{3}{4}$ to 1 ounce of chloral intravenously half an hour before administering the serum, or the chloral may be given intracæcally through a cannula, to which is attached a rubber tube and a funnel; in either method care must be taken that all the chloral solution is removed from needle or cannula by the use of normal saline, before either is withdrawn. If the chloral should gain access to intercellular tissue severe inflammation will follow.

I adopted this treatment as the result of a theory I

formulated, that the cells of the nervous system, having become desensitised by the chloral, may have lost their power to hold the haptophore group of the tetanus toxin in firm combination, thereby enabling the antitoxin to dissolve out the toxin from the cells, with consequent neutralisation. In order to have success from this method of treatment, it is necessary to keep the patient in a more or less hypnotic condition for some days, at the same time giving frequent large doses of the antitoxin.

Treatment with Cerebral Emulsion.—In view of the great affinity of the substance of the central nervous system for tetanus toxin, use should be made of this substance in cases where the antitoxin is not at hand. Fresh brain can nearly always be procured from butchers' shops. An emulsion should be made with physiological salt solution, and this substance given subcutaneously. Krokiewicz recorded sixteen cases in which use was made of this preparation, an unfiltered emulsion being injected hypodermically. Of the sixteen cases, thirteen recovered, three being severe attacks.

CHAPTER V

DISEASES PRESUMABLY CAUSED BY ULTRA-MICROSCOPIC ORGANISMS

Rabies.—This is a disease which attacks the lower animals and man; in the latter the disease is called "hydrophobia."

Causation.—The actual cause is at present not known, as the causative germ belongs to the class of ultra-microscopic organisms. In the lower animals the disease is most commonly seen among dogs, although horses, cattle, and sheep are quite commonly affected.

Although nothing is known of the poisonous material which gives rise to this malady, yet experiments show that it resides in the nervous system of infected animals, and that it can be modified in various ways. Thus light, air, and desiccation rapidly destroy the virulence of rabic matter. Heat also has the same effect. Exaltation of virulence may be effected by passing the virus through a succession of rabbits. After passage through a large number of these animals the incubation period is gradually shortened from about three weeks to a constant

period of six or seven days. Pasteur calls virus of this degree of virulence "virus fixe."

Antirabic Vaccination: Pasteur's Vaccine.—Pasteur discovered that by drying the spinal cords derived from rabid animals for varying periods of time he could prepare a series of viruses of graduated strengths. Thus, if such a cord is dried for fourteen days it loses all its toxic potency; if it is submitted to this process for only three to four days the virulence is but little reduced.

Immunity to rabies, as to other infective diseases, can be induced by injecting at first minute doses of the organism or toxin, and gradually increasing the doses until at last quite strong virus can be used. Graduation of the dose is effected by taking equal quantities of nerval matter from spinal cords which have been dried for varying lengths of time. The actual vaccine consists of a small quantity (2 to 3 mm.) of the cord of a rabbit which has been killed by inoculation with the "virus fixe." This is rubbed up into an emulsion with 5 c.c. of sterile broth or salt solution, and about 3 c.c. of the resulting fluid is injected. A cord dried for fourteen days is the first used; on succeeding occasions emulsions of less attenuated virus are used, until finally a portion of a spinal cord dried only for three or four days is used.

If bites from a rabid animal occur about the head, a more rapid form of vaccination should be em-

ployed. This is known as the "intensive" treatment. It is necessary to employ this latter method when wounds occur in the region of the head, on account of the shorter period of incubation in these cases. In the "intensive" treatment, cords dried for three or four days should be employed on the seventh day, instead of the ninth or tenth day, as in the ordinary treatment.

The technique of this vaccination varies in different countries. Thus, the Italian method of Tizzoni and Centanni is to treat the spinal cord with gastric juice, which has an attenuating effect on the virus.

Hogyes, in Buda-Pest, merely dilutes an emulsion of virulent material to different degrees, using a high dilution for the first injection, and gradually raising the concentration on succeeding days. The theory underlying this procedure is that the usual method of attenuation by drying alters the quantity but not the quality of the virus; in other words, it kills a certain proportion of the poisons, so that a smaller number of them are injected at a dose, but it does not alter their virulence. Hence the same results may be attained by simple dilution. By this last method a vaccine can be prepared much quicker than by the original method of Pasteur.

Site of Injection.—The immunising injections can be made subcutaneously in the neck in horses, and in dogs in the region of the abdomen; it is necessary to

avoid injections in the regions of nerves, as these may be injured. If a more rapid protection is desired, filtered emulsions may be given intravenously.

Serum Treatment: Antirabic Serum.—The serum of animals immunised by the Pasteurian method is capable of neutralising the virus of the disease. It is impossible to say whether this serum is antitoxic or germicidal, on account of the want of knowledge of the causative factor.

Tizzoni and Centanni recommend this serum as a protective against the disease in animals and persons who have been bitten, instead of the Pasteurian treatment. They consider this method is quicker and equally certain. The serum is prepared by inoculating sheep with rabic material, attenuated by the "Italian method." The fresh serum is dried over sulphuric acid, and preserved in this form indefinitely. Doses of from 20 to 40 c.c. should be used, given in three injections—one-half first, then the remaining half in two doses at intervals of three days. The amount of serum above is advised for cases which come under treatment within the first four days after the bite. For cases seen within the fourth and fourteenth day the amount of serum should be doubled, and very large quantities should be given in cases with the bites about the head. In veterinary patients which have been exposed to the virus of rabies, serum should be first used, followed by vaccination, and the animal kept under close observation until the probable incubative period has passed. Antirabic virus

and serum are stocked by the various institutes for antirabic vaccination. Besides the Paris Pasteur Institute, there exist institutes at Lille, Marseilles, Montpellier, Lyons, and Bordeaux in France, at Berlin, Vienna, Buda-Pest, and Kasauli in India.

In cases of bites by rabid animals, resource should be had as soon as possible to antirabic inoculation. It is important that this should not be delayed. There is practically no danger in the procedure. Many valuable animals can be saved by this treatment instead of being destroyed, as has been formerly done.

Hog Cholera (Swine Fever).—A very common disease of swine; in the United Kingdom it is one of the scheduled diseases.

Etiology.—An ultra-visible virus. Until the year 1904 this disease was thought to be caused by the *B. cholerœ suis*. However, in the above-mentioned year Schweinitz and Dorset proved the disease to be due to a filterable virus. The *B. cholerœ suis* is probably a secondary invader. In this country treatment of the disease is not permitted, and, for some reason unknown to me, prophylactic measures are not adopted either. It has been conclusively proved by the great majority of American veterinarians that in young animals an active immunity to this disease can be established. Animals that have recovered from an attack of the disease are thereafter immune, and it has been shown that their blood has some

immunising power when injected into other susceptible animals. Practical methods of immunisation were developed through the work of Dorset, McBryde, and Niles of America. The method devised by them is as follows: It is necessary to start with an animal that has recovered from the disease or has been already immunised. This animal is hyperimmunised by the repeated injections of virulent blood. Such blood must be of a strain of known virulence, established by tests. This hyperimmunised animal is then bled, the blood defibrinated and preserved by the addition of $\frac{1}{2}$ to 1 per cent. carbolic acid. This serum should be capable of protecting an animal, in doses of 15 c.c., against an injection of 1 to 2 c.c. of virulent blood.

The best results, however, have been obtained by the serum simultaneous method. Here a dose of virulent blood—1 to 2 c.c.—is given together with 20 c.c. of serum. At the present time there are numerous American firms which supply standard virulent hog cholera virus, together with the immune serum. This does away with the disadvantages which were associated with the use of virulent blood, as the blood did not always contain the same degree of virulence; furthermore, it is much more practicable. The serum simultaneous method of vaccination results in the development of an active immunity, which lasts from six to twelve months, or perhaps longer.

In this country the mortality from swine fever runs

into thousands every year, and it is inconceivable why this preventive treatment, so highly successful in other countries, is not practised. Statistics show that in herds where the mortality had been as high as 80 to 90 per cent., it was reduced to 5 to 10 per cent. by the serum simultaneous vaccination. The use of serum alone only confers an immunity lasting from four to six weeks, and is only to be recommended in an actual outbreak of the disease.

Canine Distemper.—Distemper is an acute, contagious, infectious disease of young carnivorous animals.

Etiology.—The disease is due to an ultra-visible virus, as shown by the experiments of Carré; secondary lesions are caused by invading organisms, chief of which are *B. bronchisepticus*, streptococci, and staphylococci.

Recovery from the disease confers an immunity.

Prophylaxis. — An antidistemper serum has been prepared from the blood of horses hyperimmunised against virulent strains of the *B. bronchisepticus*, isolated from acute cases of canine distemper. As this serum is only specific for the *B. bronchisepticus*, and as the organism is a secondary invader, it cannot be expected to have an immunising effect against the primary factor of the disease. In practice this serum is more or less useless as a preventative, but it has a decidedly beneficial influence on the course of the disease, inasmuch as it prevents the

secondary lesions for which the *B. bronchisepticus* is responsible. Dose of serum is 10 to 30 c.c., repeated each twenty-four hours when used as treatment.

Vaccination.—Vaccines have been prepared for active immunisation against distemper. They consist generally of a mixed infection vaccine, prepared from strains of the *B. bronchisepticus*, streptococci, and *Staphylococcus albus* and *aureus*. Dose 2 c.c., following an initial injection of antiserum. As in the case of the serum, this vaccine does not prevent the disease, and as it is a stock vaccine, it cannot influence the course of the disease very much owing to the different degrees of virulence of the strepto- and staphylococci group of organisms. In some cases where good results follow from the use of stock vaccines in diseases caused by these organisms, it may happen that the organisms responsible for the disease chance to be the same strain as those from which the vaccine is prepared, hence the beneficial results obtained from this particular vaccine.

Up to the present, no sera or vaccines have been prepared which can to any extent be relied upon to protect an animal from distemper; but by the use of the serum a protection is given to the animal from secondary infection by the *B. bronchisepticus*, and in some cases the vaccine has the same effect in protecting the animal against the invasion of the strepto- and staphylococci. Hence it is advisable to employ the serum, or the serum and vaccine combined, where young animals are exposed to distemper infection,

such as for hound puppies or in any condition where a number of young dogs are liable to congregate; for even though actual protection will not be afforded, should the animal get the disease, it generally does so in a mild form if the serum or vaccine has been previously employed.

Foot and Mouth Disease.—In the British Isles this is a scheduled disease, therefore it is not allowed to be treated.

Etiology.—An ultra-visible virus. This disease is highly contagious, and attacks bovines, sheep, goats, swine, deer, and occasionally horses, dogs and man. In countries outside the British Isles this disease is treated, but so far no serum or vaccine has been prepared which can be relied upon to give immunity. Recovery from the disease gives active immunity for indefinite periods corresponding with the severity of the attack. In countries where the disease is more or less constant, immunisation is accomplished by the deliberate production of the disease; the immunity so produced, as a rule, lasts for a long time.

Cattle Plague or Rinderpest.—In the British Isles this is a scheduled disease.

Etiology.—An ultra-visible virus. The disease is chiefly confined to cattle, rarely occurring in goats and sheep.

Immunity.—Animals which recover spontaneously from the disease are highly immune, and the blood

has some power of passive immunisation when injected into another animal. Injections of the bile from animals having had the disease has been advocated by Koch, and extensively practised in South Africa, with good results. Immunisation by the use of hyperimmunised serum, prepared from animals which have recovered from the disease, has been developed by Kolle and Turner. Twenty c.c. of this serum should protect an animal against infection of 1 c.c. of virulent blood. An injection of 50 to 100 c.c. of this serum will protect an animal against infection for a period of two to four months usually. A more permanent immunity may be established by the use of the serum simultaneous method. The animal is injected on one side with 8 to 25 c.c. immune serum and on the other with 1 c.c. of virulent blood. Some animals react by a distinct fever, others show no effect; the latter are rendered immune for several months only, while the former for a much longer period.

Pleuro-Pneumonia Contagiosa of Bovines.—This is a contagious disease of cattle and other bovines. The disease is scheduled in the British Isles. It is very prevalent in Europe and other countries.

Etiology.—The causal organism is doubtless a bacterium that is just at the limit of visibility. In suitable fluids it may be observed, under very high power of microscope, as a tiny motile point.

Immunity.—Recovery from the disease results in

a relatively permanent immunity. Immunisation against the disease by vaccination with serum from the pleural cavity of infected animals has been practised. The material is injected subcutaneously. Its use is attended with danger, as up to 5 per cent. or even more of those vaccinated have been killed by the vaccine. Nocard and Roux have advised vaccination with pure cultures, and claim to have secured more favourable results than by the older methods. Nocard has also produced a curative and prophylactic serum by the hyperimmunisation of animals by the injection of increasing doses of pure culture until 6 litres have been used. In doses of 40 c.c. it serves as an efficient prophylactic, and in larger amounts as a curative agent in the early stages of the disease.

Cow-Pox (Variola Vaccina).—Cow-pox, the relation of which with human pox has already been recognised by Jenner (1796), occurs usually only sporadically, or as an enzootic confined to individual herds. In most cases it may be attributed to an infection of true pox or vaccination pox of man. It is a mild disease, attacking chiefly the teats of cows, and causes considerable economic waste in dairy herds, owing to the loss of milk caused by the difficulty of milking the infected animals. Under medicinal treatment it may be often difficult to eradicate, as new animals, when introduced into the herd, become affected.

Immunity.—A simple means of establishing immunity in the herd and in all newcomers is to vaccinate the animals with calf lymph used for man. The vaccination is carried out on the perineum by superficially scarifying the skin. Nodules develop on the affected cows, while on the healthy animals typical pox pustules appear, and these animals do not contract the infection. Personally, I have had very excellent results from this procedure in herds where the disease had been prevalent for years.

Horse Sickness of South Africa.—This disease is characterised as an acute or subacute disease of solipeds, and appears in epizootics during the hot months of the year. The principal lesions are œdematous swellings and hemorrhages of the internal organs.

Etiology.—McFadyean, in 1900, was the first to show that this disease was caused by a filterable virus.

Immunisation against the disease may be established by the use of serum from hyperimmunised animals, or by the use of immune serum and virulent blood. This latter method is advocated by Theiler; he injects 300 c.c. immune serum intravenously and $\frac{1}{2}$ c.c. virulent blood subcutaneously. Animals thus treated will show a rise of temperature, upon which a second injection of 50 to 100 c.c. serum is made.

CHAPTER VI

ACUTE DISEASES PRODUCED BY MICROSCOPIC ORGANISMS

Anthrax (Splenic Fever).—This disease is also known in cattle as "splenic fever." In man the disease is termed "malignant pustule" or "wool-sorter's disease." The animals most commonly attacked are cattle, sheep, swine, and more rarely horses and man.

Etiology.—The *B. anthracis.* In cattle the disease is generally in the form of an acute septicemia, and in the British Isles it is notifiable. It is prevalent in all countries, and in cattle, among which it is commonest, on account of its acute nature, there is very little chance of treatment, the animal generally being found dead. Prophylactic measures, however, can be adopted with very real success.

1. *Immunity.*—Active immunisation by vaccination has been extensively practised. The first method developed was that of Pasteur, and is still the one most commonly used. The organism is grown at a temperature of 42° to 43° C. for varying lengths of time. The pathogenicity gradually decreases until injections no longer kill the rabbit; longer growths attenuate it until the guinea-pig is not susceptible,

and finally even the mouse will not succumb. The exact length of time required for attenuation in each instance can be determined only by experimentation. Pasteur uses two vaccines, the first prepared as above, the second attenuated to a less degree. The second vaccine is injected ten to twelve days after the first. The immunity reaches the necessary degree in about ten days after the second inoculation, and lasts about one year. Statistics show that this method of vaccination has been thoroughly satisfactory.

2. *Immunisation with Immune Serum.*—The serum is obtained from hyperimmunised animals. This serum gives a passive immunity, and has also curative effects in cases where the disease is localised, and if given shortly after infection, prevents the development of the disease. Such immune serum also renders good service in practice, especially in cases where infection threatens or where anthrax has already appeared in the herd. The action of the injected serum becomes effective in a few hours. However, this passive immunity only lasts from one to two weeks. It is therefore advisable in cases of continued danger of infection to render the animals actively immune.

3. *Immunisation with Immune Serum and Cultures.*—This method is recommended by Sobernheim for a lasting, combined, passive and active immunisation. Cattle and horses are injected on one side of the body with 5 c.c., calves with 3 to 5 c.c., and sheep

with 4 c.c. of immune serum; five minutes later they are injected on the opposite side with 0·5 or 0·25 c.c. of an attenuated culture, washed in salt solution, the degree of virulence of which corresponds with Pasteur's second vaccine. This method has the great advantage of only requiring a single treatment of the animals, and the results obtained from it are quite satisfactory. In Argentine and Uruguay this method of vaccination was employed from the spring of 1904 to September, 1905, on 140,000 cattle, 30,000 sheep, and 2,000 horses. According to Sobernheim no fatalities resulted from the vaccination, and almost everywhere a complete eradication, or at least a marked restriction, of anthrax was noted. In this country, where outbreaks of anthrax are continually occurring, I consider it the duty of all practitioners to acquaint their clients with the protection afforded to their stock by vaccination. Every stock-owner knows the results obtained from black-leg vaccination, but few are aware that almost the same protection can be gained against anthrax by vaccination against the disease.

Black-leg, Black-Quarter, Quarter Ill.—Black-leg is an acute infectious, but not contagious, epizootic disease of cattle, and exceptionally of swine.

Etiology.—The black-leg bacillus, *B. Chauveaui.* Black-leg is a very common disease, occurring chiefly among cattle. At the present time there are available at least five biological products for the prevention and cure of this disease.

1. Two inoculations with attenuated virus (method of Arloing, Cornevin, and Thomas). A vaccine is prepared from diseased powdered muscle by attenuating the virus through heating to a high degree of temperature. For this purpose the diseased part of the muscle is ground in a mortar with some water, and dried in a thin layer at 37° C.; then the dry mass is mixed with two parts of water, and one half heated at 100° to 104° C., the other at 90° to 94° C. for seven hours. The former makes the first weaker vaccine, the latter the second stronger one.

The dried material is either compressed in one-dose tablets or pellets, or threads are soaked in a suspension of the attenuated organisms. The threads are collected into small bundles, each bundle constituting a dose. The pellets are injected under the skin by means of a hollow needle fitted with a plunger; the thread form is introduced into the skin of the tail by means of a special needle provided with a notch to hold the threads while being inserted. The second inoculation is made ten to twelve days after the first one. The immunity reaches the necessary degree in eight to ten days after the second vaccination. Until then, however, the animals have a lower resistance against the natural infection (negative phase).

2. One inoculation with attenuated virus. According to Kitt, a powder prepared from diseased muscles, after having been heated for six hours in dry air at 85° to 90° C., or, better still, in live steam at 97° C., gives a suitable vaccine. One injection of such

vaccine into cattle immunises them sufficiently and lastingly. This method is conducted in the same way as that of Arloing, Cornevin, and Thomas's. By either method the immunity established is believed to last for eighteen months.

3. Black-leg aggressin. A germ-free product, prepared from the tissue juices of calves previously inoculated with the black-leg organism. This confers a rapid and lasting immunity. Dose is 5 c.c., given subcutaneously.

4. Black-leg filtrate, also known as artificial aggressins. This is a germ-free cultural product, the dose and method of application being the same as for the aggressins.

It has been conclusively proved that the immunity following both the aggressins and filtrate, which in themselves are complex and not thoroughly understood bacterial products, is strong, lasting, and satisfactory.

5. An immune serum has been prepared from hyperimmunised animals, the animals used being horses or cattle. The use of this serum is becoming extremely popular as a curative for the disease, as results show that this product is quite efficacious in the treatment of infected animals.

Swine Erysipelas.—Swine erysipelas is an acute septicemic infectious disease of young pigs, caused by a very fine rod-shaped bacterium, the *Bacillus erysipelatis suis.*

This is quite a common disease, and occurs generally throughout, and is usually prevalent in, the infected region in summer. Mortality from this disease is as high as 80 per cent. Some of the acute cases recover completely; the chronic cases nearly all end fatally.

Prophylaxis and Treatment.—One attack of the disease confers immunity.

1. Protective inoculation with attenuated cultures. (Pasteur's method). Pasteur showed in his experiments that the passage of erysipelas virus through the body of rabbits increases its virulence for those animals. On the other hand, it reduces it for pigs, so that the inoculation of such virus in the latter animals causes only a slight febrile disease. Virus attenuated up to a certain degree retains this degree of virulence, even if transferred from the rabbit blood to a suitable artificial medium, and if further cultivated at body temperature. A bouillon culture attenuated by the above method produces the vaccine, which is injected subcutaneously into young pigs in the form of a weaker and stronger modification. The efficacy of Pasteur's protective inoculation has been positively established.

Dose: 0·12 c.c. of the weaker vaccine is given subcutaneously, and twelve days later 0·12 c.c. of the stronger is given. In pure-bred pigs the above method of vaccination is sometimes attended with serious affections, but in the coarser breeds the results have been highly satisfactory.

2. Serum simultaneous method (method of Lorenz and Leclainche).

The serum is obtained from hyperimmunised animals, and this is used in conjunction with virulent cultures. The advantage of this method over Pasteur's consists in the development of the passive immunity immediately after the serum is injected. This method is also attended with some of the dangerous results seen in Pasteur's method.

Dose: 1 c.c. of a bouillon culture and 9 c.c. of immune serum are injected simultaneously. The serum and culture are well mixed before injection. The injections are made on the inner surface of the thigh.

3. Passive immunity is established, and lasts for sixteen to eighteen days, by the use of immune serum. This method has the advantage in cases where the infection has occurred, and in animals open to infection, as the necessary immunity is rapidly established. Serum is also used in treatment of the disease, with considerable success if given at the onset.

Doses: 10 to 30 c.c. given subcutaneously for treatment, and repeated every six hours. For passive immunity, 10 to 15 c.c. is sufficient.

Hemorrhagic Septicemia or Pasteurellosis. — Under the collective name of "hemorrhagic septicemia" are included all those diseases which are produced by varieties of the *Bacillus bipolaris septicus*, and in which the acute cases are characterised by

manifestations of a general infection and hemorrhagic inflammatory processes of the internal organs. The bipolar bacilli are very widely spread, and may produce disease in any species of animal, and more especially the domesticated ones. Originally saprophytes, they occur in nature in the soil, in stagnant water, on various plants, and in all kinds of dead organic matter. Under normal conditions they do not manifest pathogenic characteristics. Under certain conditions, which at present are still unknown, they become very virulent, and then may attack the healthy tissues of the animal body. On the other hand, their pathogenic action is favoured by all those influences which weaken the body resistance. Some of the organisms which have become parasites and pathogenic, after leaving the body again return to their saprophytic harmless existence, but in the majority of cases, once they have gained a parasitic existence, their virulence remains exalted for the particular species of animal they have infected, and by their dissemination are liable to spread the disease among the particular species of animal. This relative virulence may even increase for a time in the course of further generations, or may become constant. More frequently, however, it diminishes again after a certain time. These characteristics of the bipolar bacteria explain the variation which is observed in the appearance and spread of the diseases belonging to this group. Commonly these diseases appear in a locality periodically, without any apparent connection

to which the introduction could be traced. In such cases the outbreaks can be explained by a sudden increase in the virulence of the bacteria which are present in the soil or in the healthy animal, favoured by a decreased resistance of the animal body to infection.

The chief diseases resulting from infection with the bipolar bacteria which occur in the British Isles are hemorrhagic septicemia of cattle, fowl cholera, and swine plague. A bipolar bacillus, the *B. suisepticus*, is a very common secondary invader in swine fever. It is this organism that is also responsible for swine plague. Septicemia hemorrhagica is a common disease in this country. Personally, I have experienced several outbreaks, all of which occurred in the summer, and especially when the drinking water was low. I believe that many of the outbreaks of so-called bracken poisoning are in reality outbreaks of this disease. Clinically the symptoms of septicemia are practically the same as those described for bracken poisoning.

Etiology.—*B.* (*bipolaris*) *bovisepticus.*

Prophylaxis.—As the disease in cattle is generally very acute, animals often die within twelve hours of the appearance of symptoms; therefore it is evident that prophylaxis is the better course to adopt. At the present time there are three methods of vaccination in vogue:

1. Vaccination with dead cultures, or "bacterins," as they are now commonly called.

2. Use of attenuated cultures.

3. Use of cultures and immune serum.

Vaccination by any of the above methods establishes active immunity.

Passive immunity is given by the use of immune serum, and this method is indicated to immunise the in-contact animals in an actual outbreak.

All the organisms belonging to the bipolar group have a close relation, inasmuch as the serum prepared from one species is not strictly specific for that species, but has immunising properties for all the varieties. However, the immunising properties of the serum are most marked for the variety of organism from which the serum is prepared. In the early stages of the disease, if serum is given in doses of 50 to 100 c.c. or more, good results will follow. The serum should be given intravenously. The serum now in use is generally polyvalent—that is, several virulent varieties of the bipolar bacilli are employed in hyperimmunising the animal which produces the serum. A similar polyvalent vaccine has been prepared by Lignières, and has met with much success in practice, especially in establishing immunity against the acute forms of infection. Statistics show that the most favourable results have been obtained from vaccination by the serum simultaneous method. The vaccine employed is generally polyvalent.

Dose: 2 c.c. of vaccine and 20 to 40 c.c. of the immune serum.

In actual outbreaks the serum may be given in

doses of 40 to 60 c.c., and immunity is sufficiently established within twelve hours to prevent infection and last for about four to five days. Later the vaccine should be used to establish an active immunity. Dose of the attenuated culture vaccine is 2 c.c. Dose of bacterins 2 to 4 c.c., given subcutaneously.

White Scour of Sucklings.—White scour of newly born animals, especially calves, is an acute contagious infectious disease, infecting the animals in the first days of their lives, and is observed usually as a stable infection characterised by profuse diarrhœa and rapid exhaustion.

Etiology.—The *B. coli communis* is the organism most commonly attributable for the disease. However, such organisms as the *B. aerogenes*, paracolon bacilli, also streptococci, *B. abortus*, and the *B. enteritidis* are stated to be causative agents. A fatal disease of new-born infants, known as "Wenckel's disease," is also caused by colon bacilli infection.

The colon bacilli are common inhabitants of the intestines of man and animals, and under ordinary conditions these organisms are quite benign. For some unknown reason, however, their virulence may become exalted, and then in young animals they give rise to serious infections. Once their virulence has been exalted it remains so for several generations. A remarkable fact is the strict specificity of virulence of the colon bacilli species; this specificity not only applies to species, but to in-

dividuals of the same morphological species. The prevalence of disease in new-born animals caused by the colon group of bacteria may perhaps be accounted for by the fact that the serum of new-born animals does not contain any free antibodies for these organisms, whereas in older animals the serum does. Thus the normal serum of an adult animal will agglutinate this bacillus in dilutions of 1 : 10 or 1 : 20 —a phenomenon possibly referable to its habitual presence within the body.

On account of the strict specificity of each strain of the colon bacillus, it is extremely difficult to prepare a serum or vaccine containing universal immunising properties against this group; also, as the disease is commonly caused by other varieties of bacteria, it makes the preparation of such serum or vaccine for practical purposes still more difficult. However, an immune serum of a polyvalent character has been prepared by Jensen, and, according to this author, very satisfactory results have been obtained from its use. Mixed bacterins are now commonly in use, prepared from the *B. coli communis*, paracolon bacillus, and the *B. abortus*. According to statistics, very favourable results have been recorded from the use of both the serum and vaccine. The serum should be given in doses of 20 to 30 c.c. immediately after birth to animals born on infected premises. As a curative, where the disease has appeared, from 50 to 100 c.c. should be given. The passive immunity so established may be prolonged by the subsequent

administration of the mixed bacterin. Dose of the bacterin is 2 c.c.

Remembering the strict specificity of the various strains of the colon bacillus, it is very hard to believe that good results can follow from the use of stock vaccines or serum; however, as such strict specificity does not apply to the other organisms regarded as accountable for white scour, the good results recorded from the use of stock vaccines and serum may be referred to the immune bodies provoked against these latter organisms. A method of prophylaxis against white scour I have personally adopted with very good results is as follows: From an animal which has died of the disease I have an autogenous vaccine prepared; the bacteria chiefly found are the colon bacillus and, in some cases, the paracolon bacillus. I presume that it is this particular strain of organism which is virulent on the particular premises where the infection occurs. This vaccine is used to vaccinate the pregnant cows on the premises. The vaccination should be carried out during the seventh and eighth months of pregnancy, as if left till later the cow may abort. Three injections of the vaccine are made at intervals of ten days. The first injection contains 10 million dead bacteria, second injection 25 million, and third injection 50 million. My idea is to actively immunise the mother against the particular strain of organism responsible for the disease, and thereby transmit the protective bodies to the fœtus by way of the maternal circulation. By this method I have completely eradicated

white scour from eight dairy farms where the disease was very prevalent. The disadvantage of this method is the preparation of a different vaccine for each different farm, but the results obtained far outweigh this.

Pyosepticemia of Sucklings (Joint Ill, Navel Ill).—Joint ill of sucklings is an acute, sometimes chronic, contagious infectious disease of the newly born animals, generally occurring in the first few days of life. It is characterised by purulent inflammation of the joints and general pyemic manifestations. In some very acute cases the manifestations are septicemic. It develops in some cases as a result of umbilical infection, occasionally as an intestinal infection, and commonly as an intra-uterine infection of the fœtus.

Etiology.—In foals the following organisms are accountable for the disease:

1. Streptococci; these bacteria cause about 50 per cent. of joint-ill cases.

2. *B. nephritidis equi*, 21 per cent.

3. Organisms of the *B. coli communis* type, 21 per cent.

4. Miscellaneous organisms, chief of which is the *B. abortivo equinis*, 5 per cent.

In calves the causative agents are chiefly:

1. *B. bipolaris septicus.*

2. *B. coli communis.*

In the latter animals joint ill and white scour are

commonly associated, and, according to some authors, the colon bacilli prepare the field for the pathogenic bipolar bacilli, the latter giving rise to the septicemic infection.

Prophylaxis.—There are various brands of serum on the market, all lauded as giving protection against joint ill. When we consider the variety of organisms responsible for the production of the disease, we can easily imagine why such futile results are obtained in practice by the use of a serum prepared from one particular organism in giving protection against joint ill. A serum, to be of any use, should be polyvalent—that is, prepared from all the organisms that are found responsible for the production of the disease. In practice such a serum is found to be poor in antibodies specific for each organism from which it is prepared. However, the use of a polyvalent serum offers a better chance of affording protection, especially if given immediately after birth, than any of the monovalent serums.

Vaccines have also been advocated as giving protection, and lately McFadyean has advised the use of a polyvalent vaccine prepared at his Research Institute as a preventative. It may be used in the following ways :

1. To vaccinate the mares before foaling.
2. For vaccination of foals after birth.
3. For treatment of foals attacked with the disease.

In considering the use of vaccine, we must remember that in most cases it takes from six to twelve

days for a sufficient immunity to be acquired to resist the infection against which the vaccination is made; also that immediately after the vaccine is inoculated there is a decreased resistance of the animal's body—the negative phase. This may last from twenty-four hours to several days, Now, in the vast majority of cases of joint ill the disease appears within the first week after birth, and it may appear in a very acute form and cause death within twenty-four hours after birth. Having these facts in view, it does not seem possible that postnatal vaccination can be of any service in preventing the disease. If, however, the vaccine was used in conjunction with polyvalent immune serum, better results might be hoped for, especially in the more chronic form of infection. The best results should be obtained from treatment of the mares during pregnancy with a vaccine as advised by McFadyean, and which corresponds to the method adopted by me in immunisation against white scour, and from which I had such favourable results (see White Scour).

Treatment.—Up to the present serum treatment has not been successful. In all kinds of cocci infections antiserum as a curative has been a failure. In an article appearing in the *Veterinary Record*, March 19, 1921, by Edwards, he mentions, with reference to a serum supplied by the Royal Veterinary College, London, "that many individual practitioners formed the opinion that this serum exercised a markedly beneficial effect." At the same time there

is no proof that the beneficial effects noticed in treatment by serum were afforded by the specific antibodies present in the serum. I contend that normal serum would be quite as beneficial. In human medicine antidiphtheritic serum has been used in streptococci infections in many instances with better results than from the use of antistreptococci serum. In this case the benefit observed cannot be referred to any specific antibodies for the streptococci present in the diphtheritic serum. How, then, does this serum, in which specific antibodies are absent, exert its curative effect? This matter is fully discussed in the chapter on immunity. I believe it is the free complement present in the fresh serum which combines with the antibodies already present in the animal's system, thereby causing the activation of the latter, that exerts this curative action.

Vaccines in Curative Treatment.—Except in chronic cases, this method would appear to be useless, if not positively harmful; good results, however, should be hoped for from the use of a polyvalent and, if possible, autogenous vaccine in treating chronic cases of this disease. Personally, I would recommend the method described on p. 85, not only in this disease, but in all diseases of a pyosepticemic and septicemic nature.

Purpura Hemorrhagica (Petechial Fever).—Purpura hemorrhagica represents an acute non-infectious disease or process, probably caused by septic bacterial intoxication, possibly the same as, or similar to, the

anaphylotoxins of Friedberger, or a sequel to a specific infection. It is principally characterised by extensive œdematous infiltrations of the subcutaneous connective tissue and by hemorrhages in the swellings, as well as in the mucous membranes and in the internal organs.

Prophylaxis and Treatment.—No attempt at immunisation against this disease has been made.

In treatment various authors, on the presumption that this disease was a bacterial infection or sequel to a bacterial infection, due to the cocci group of organisms, have used antistreptococci serum, and, according to reports, with favourable results. However, Cadeat obtained equally good results with normal blood serum. Treatment of this disease by the use of vaccines has not been attempted.

Strangles.—Strangles is an acute contagious infectious disease of horses, in the course of which catarrhal symptoms of the upper air passages develop in association with suppurative inflammation in the adjoining lymph glands, and sometimes in a generalised form in more distant lymph glands.

Etiology.—The *Streptococcus equi.* Horses when recovered from this disease are immune to subsequent natural infection. This immunity lasts for several years.

Immunisation and Serum Treatment.—As in all other streptococci infections, serum treatment is of very little use as a curative; however, it is very useful

in producing passive immunity in the animals which have not become affected, if it be prepared from the particular strain of organism responsible for the outbreak of the disease. Thus if the serum of animals recovered from the disease be used to protect the healthy in-contact ones, good results can be hoped for. However, this immunity is of short duration.

Vaccines prepared from killed cultures of streptococci have been used to give active immunisation, but the results are very indifferent. An autogenous vaccine should give much better results in treatment, as obviously an outbreak in a stable is caused by the same strain of organism; this latter vaccine is especially useful in chronic conditions or in attacks which run an irregular course, such as abscess formation in regions remote from the primary seat of the affection. A method now extensively used in human practice, both in the treatment of affections due to the various pyogenic cocci and for production of active immunisation against other infections, is the use of sensitised vaccines. An autogenic vaccine is prepared, and either before or after its sterilisation the bacterial emulsion is mixed with its specific antiserum, the antiserum being previously heated to 56° to destroy the complement. This mixture is then allowed to stand for twelve hours, when it can be shown that the bacilli have entered into combination with their specific amboceptor. The bodies of the bacteria are then washed free from serum in several changes of physiological saline solution and standardised in the

usual manner. Besredka, who first introduced this method in vaccinating against plague, cholera, and enteric fever, claims that with these sensitised vaccines active immunity is produced in the inoculated individual almost immediately. Broughton-Alcock uses sensitised living vaccines of the pyogenic cocci in the treatment of streptococci and staphylococci infections with remarkable success. This method has now been extended to include the treatment of numerous different bacterial infections in the human subject. It is worthy of trial in veterinary practice, where it is so much easier to obtain a homologous specific serum than in human practice. It should be admirably suited to give active immunity to healthy animals in an outbreak of strangles.

CHAPTER VII

CHRONIC INFECTIOUS DISEASES

Tuberculosis. — Tuberculosis is a chronic contagious infectious disease of man and domestic animals. It is characterised anatomically, in its incipient stage at least, by small non-vascular nodules known as tubercles, which have a tendency to undergo cheesy degeneration.

Etiology.—Koch's *B. tuberculosis* or one of its varieties.

Immunisation. — So far no practical attempt at immunisation against tuberculosis has been made in the lower animals with any success. Behring, in 1901, observing the varying pathogenic action of human and bovine tubercle bacilli, declared that by treating cattle with the less virulent human tubercle bacilli they would be protected against the more actively virulent bovine type, and thus also against natural infection with tuberculosis. On the basis of this assertion several methods of immunisation for cattle have been recommended and actually carried out in practice. However, satisfactory results were not obtained except in cases where the most strict hygienic and prophylactic measures were

observed at the same time. As these latter measures are in themselves effective, the beneficial results obtained could not solely be attributed to the immunisation.

Double Immunisation with Human Tubercle Bacilli (Behring's Method).—The immunising material consists of nearly free virulent human tubercle bacilli, dried in a vacuum, and injected into the blood circulation of calves.

The first tests conducted after these treatments showed that two intravenous injections of bovovaccine, or of any fresh culture of the human type of tubercle bacillus, into cattle resulted in a considerable and immediate increase in their power of resistance to artificial infection. Further experiments, however, showed that resistance thus artificially increased was of short duration, suffering considerable reduction at the end of one year, and disappearing altogether at the end of one and a half years. The results of the practical application, on the other hand, of this method showed that it had no noticeable effect on the improvement of the health of infected herds. A serious objection to this plan, apart from the trouble and expense of yearly vaccination, lies in the fact that the bacilli introduced by the act of vaccination remain alive and active in the bodies of the animals for a long time, thus making the slaughter of such animals for food a procedure of doubtful propriety.

Koch introduced a method similar to the above,

but only used a single vaccination. However, the results were no better than those of Behring's methods, and the same objections applied.

Recently Calmette, of the Pasteur Institute, has evolved a method of vaccination, the vaccine being prepared from attenuated bovine tubercle bacilli. Calmette demonstrated that if the bovine tubercle bacillus was grown for several generations on a glycerinated media, containing ox-bile, an attenuated type of the bacillus could be produced that was avirulent for the ox. Moreover, not only would the animals withstand a large intravenous dose of these bacilli without any lesions of tuberculosis ensuing, but in the case of cattle, after a period of thirty days, there was sufficient immunity established to withstand intravenous test inoculations with living virulent tubercle bacilli. According to experiments conducted by Calmette with this method, animals which had been vaccinated yearly and were exposed to natural infection continuously for nearly three years, at the end of this time were perfectly free from tuberculosis, whereas control animals had all become affected. Calmette draws the following conclusions from his method of vaccination:

1. That the avirulent strain of tubercle bacillus behaves as a true vaccine in that, when inoculated intravenously into cattle in suitable doses, it confers a definite immunity, not only against experimental inoculation, but also in the face of natural infection by close cohabitation in infected buildings.

2. This tolerance, depending, he believes, on the presence of the avirulent bacilli in the system, does not last more than eighteen months after a single vaccination, but it may be maintained by annual revaccinations, which are harmless in themselves. It is only by vaccination on a large scale that the practical value of this method can be accurately estimated, and, if then found effective, here, then, would be a valuable step towards the protection of our animals against the ravages of tuberculosis.

Tuberculin. — Koch found that by growing the bacilli from six weeks to two months in flasks containing slightly alkaline veal broth, to which a percentage of peptones and of glycerine had been added, and freely supplying the cultures with oxygen throughout, it was possible to obtain a fluid containing some, at any rate, of the toxins produced by the organisms. By passing this through a porcelain filter the bacteria were removed, and a solution of the poisons remained. This was concentrated by evaporation to one-tenth of its bulk, and to the fluid thus obtained the name "tuberculin" or "old tuberculin" was given. There were great hopes that this would have a curative effect on tuberculosis, but these hopes were dashed to the ground when further experience was gained as to the limitations of tuberculin and the inconstancy of the good results produced. In human medicine numerous modifications of Koch's original tuberculin have been employed in the treatment of tuberculosis, but they have all

proved ineffective. In veterinary medicine tuberculin has only been used as a diagnostic agent, and in it we have a valuable preparation for such purpose at our disposal.

In addition to the above tuberculin, Koch subsequently introduced other remedial preparations of tubercle bacilli. Instead of the toxin produced by growth of the bacilli in a fluid medium, he made use of extracts of the organisms themselves. He took highly virulent cultures of tubercle bacilli, dried them *in vacuo*, and triturated them in a mortar. The resulting powder was treated with sterile distilled water and submitted to centrifugalisation. The supernatant clear, but opalescent, fluid was then removed from the débris, and 20 per cent. of glycerine added as a preservative. To this Koch gave the name of "Tuberkulin-O." The solid residue, thus freed from soluble toxins, was then dried, and the process of extraction by triturating with 20 per cent. glycerine solution, and then centrifugalising, was repeated several times, the fluid each time being preserved, and the whole finally mixed together. This mixture constitutes the new tuberculin.

Action of Tuberculin.—It is found that if a small quantity, 30 to 60 minims, of the old tuberculin is injected hypodermically into an animal suffering from tuberculosis, very definite symptoms are produced. There is a rise of temperature of varying intensity from 2·5° to 4° Fahrenheit, accompanied by dullness and appearance of illness. The mode

of production of the fever is not well understood. It cannot be due to the existence in the tuberculin of a direct thermogenic substance, as in that case normal individuals would be affected in the same way as the tuberculous. It has been suggested that in the case of the old tuberculin the fever is the result of the local inflammation excited around the lesions, but this is doubtful in view of the similar action of the new preparation, which is not followed by any such local effects. Others have suggested that the tuberculin reaction is a condition of anaphylaxis, but by a series of experiments Besredka has conclusively shown such is not the case. Ehrlich has put forward the following explanation: The normal cells of the body are not affected by this substance, nor are those which form the actual tubercular tissue. Probably the latter are habituated to the poison, as they are in close relation with the bacilli which are constantly giving rise to its formation. There is, however, a zone of cells at a certain distance from the centre of infection which have been only so far affected by the poisons of the bacillus as to be rendered unusually susceptible to their influence. When an injection of tuberculin is administered, an additional quantity of poison is brought into contact with these cells, and they are thus stimulated to react. The reaction takes the form of inflammation—the process by which dead or dying tissues are cast off from the body, as is seen in the separation of a sequestrum or a slough. This explanation is supported by the fact that animals in

advanced stages of tuberculosis, and where all the cells of the body are presumably soaked with the tubercle bacillus poisons, often fail to react to the injection of tuberculin.

Methods of Administering Tuberculin as a Diagnostic Agent: (1) *Subcutaneous Injection.*—This is the method most frequently used in this country. The ordinary dose of tuberculin for subcutaneous use is: adult cattle, 30 to 60 minims, according to the size; yearlings, 20 to 30 minims; calves under six months, 10 to 15 minims. The injection is made into the loose skin on the side of the neck, the seat of injection having been previously clipped and washed with an antiseptic. The animals to be tested should be under normal conditions as to feeding, housing, etc. Their temperature should be taken before the injection is made, and animals showing a temperature of 2·5° above normal should be left until the normal temperature is regained.

The characteristic thermal reaction for tuberculosis consists in a gradual rise of temperature beginning from the sixth to the twelfth hour after subcutaneous injection of the tuberculin, reaching its maximum from the twelfth to the eighteenth hour, thereupon falling with slight interruptions until the normal is reached. After injections the temperature should be taken at the ninth, twelfth, fifteenth, and eighteenth hour.

The difference between the maximum temperature after injection and the temperature recorded before

injection is usually from 2° to 4° F. In rare cases the fever curve just described may show variations in that it may begin sooner or much later, and then, particularly in the latter case, reach the maximum at a later hour also. Simultaneously with the thermal reaction, depression and loss of appetite are frequent, and in cows the milk secretion is generally diminished for a few days. In interpreting a tuberculin reaction the following principles are a safe guide to follow. Animals over six months of age may be regarded as tuberculous—(*a*) if the highest temperature indicated in the course of a typical reaction exceeds the highest recorded temperature before the tuberculin injection by 2·7° F. or more, or if the rise was at least ·9° F. and higher than 104° F.; (*b*) if the temperature is elevated 1·8° to 2·5° F. or exceeds 103·1° F. with symptoms of an organic reaction. Calves under six months old must show an increase of temperature over 104·9° or 105° F. to indicate a positive reaction.

The intensity of the reaction and the extent of the lesions bear no relation to each other, unless this is an inverse one in the sense that extensively tuberculous animals, hence emaciated and weak, usually react with less intensity than robust individuals in which the tuberculous process is in its first stages or more or less localised.

The animal may be considered free from tuberculosis if the rise of temperature was not more than 2·7° F. and did not exceed 103·1° F., and if the animal showed no organic reaction. Many clinicians, however, con-

sider a reaction positive if the highest temperature after injection exceeds the pre-injection temperature by 2·7° F.

(2) *Ophthalmic Tuberculin Test.*—For this test concentrated bovine tuberculin is used. A few drops of the tuberculin are dropped into the conjunctival sac, the eye is then closed and slightly massaged for about one minute. The result of the test is determined between the twelfth to the twenty-fourth hour after application of the tuberculin. The untreated eye serves as a control. Particular attention should be paid to the character of the exudate and to the appearance of the membrana nictitans. In positive reactions the latter is intensely reddened and its normally sharp border is thickened and swollen, there is lacrymation of the eye, and about the twelfth to eighteenth hour there is a production of a purulent secretion which accumulates under the nasal canthus of the eye. In healthy animals a catarrhal inflammation of the eye may be produced. This test is very useful where there is a suspicion of a previous subcutaneous injection of tuberculin having been given, as this has no effect on the conjunctival reaction, whereas in the subcutaneous test a previous injection may obscure the reaction.

(3) *Intradermal Test.*—Von Pirquet (1907) has shown that the injection of minimal quantities of tuberculin into the upper layers of the cutis of tuberculous patients produced local reddening and swelling at the point of injection. Vallée applied this diagnostic method in horses and cattle with the same satisfactory

and positive results. The scarified area into which the tuberculin had been rubbed usually showed a distinct reaction in tuberculous animals, while animals showed slight inflammatory reddening at the border of the area only in exceptional cases. This test may be applied to any part of the body, by first shaving the hair, but a convenient place for the application of the test is the hairless anal folds at base of tail. Having washed the skin, a 50 per cent. concentrated tuberculin is dropped on the part, and through the drop the skin is scarified with a sharp scalpel. The opposite anal fold acts as a control. In this test previous injections of tuberculin made a short time before may prevent the development of the cutaneous reaction.

According to collected experiences, the best results are obtained from the subcutaneous method. The intradermal test is next in value to the subcutaneous method. The ophthalmic test is very useful where there is any suspicion of a previous injection being given. This test should be employed in conjunction with the subcutaneous one.

Specific Paratuberculosis of Cattle (Johne's Disease).—This is a disease of bovines, the seat of the lesions being in the intestines, and characterised by extensive maculate swellings and reddening of the intestinal mucous membrane, clinically characterised by chronic diarrhœa and wasting of the animal. The disease is universally distributed, and occurs generally in an enzootic form.

Etiology.—A small acid-fast bacillus is constantly present in the lesions, and it is thought to be the cause of the disease. Bang has demonstrated that the disease is contagious.

Tuberculin Test.—Tuberculin prepared from the avian type of the tubercle gives a positive reaction when injected into animals suffering from Johne's disease, and is therefore used as a diagnostic agent. Bovine tuberculin does not give a reaction.

Glanders (Farcy).—Glanders is a contagious, infectious, and usually chronic disease of equines; it is communicable to man, in whom the disease is generally acute.

Etiology.—The *B. mallei.* Recovery from this disease does not give immunity, nor are there any methods known which give artificial immunity.

Mallein.—This is a substance similar to tuberculin, prepared from the *B. mallei,* and is used as a diagnostic agent for glanders. Mallein contains the toxic substance of the glanders bacillus cultivated in a liquid glycerine medium. The following are the methods employed in applying mallein for diagnostic purposes:

1. *Subcutaneous Test.*—One-half to 1 drachm of mallein is injected subcutaneously in the neck, the site of injection having been previously disinfected. Before injection the temperature of the animal is recorded. The temperature is taken at the ninth, twelfth, fifteenth, and eighteenth hour after injection.

The reaction from this method is thermic and local at the site of injection. In diseased animals the temperature is generally at its height from the twelfth to fifteenth hour, but, as with tuberculin, this is not constant, and the mallein reaction may show the same variation as the tuberculin reaction. When the reaction is positive, other symptoms are generally present, such as depression, increased respirations, and loss of appetite, as well as a more or less extensive inflammatory swelling at the point of injection. The mallein reaction is positive if the temperature after injection exceeds the pre-injection temperature by 2·7° F. or more and rises above 104° F., provided the pre-injection temperature did not indicate fever. The reaction is positive also if the increase in temperature is only 1·5° to 1·9° F., provided it is attended with a pronounced organic and local reaction; the local swelling should have a diameter of about 5 inches or more, and the lymphatics in the vicinity of the swelling appear corded and radiate outwards from the periphery of the swelling. The reaction is negative if the rise of temperature does not exceed 1° F. and no organic action occurs.

2. *Intrapalpebral Test.*—This was the method used chiefly in the French and English armies throughout the late war in testing horses for glanders. About 2 minims of concentrated mallein is injected into the palpebral border of the lower lid of the left eye. A small syringe containing eight doses, with a very fine needle, should be used. Where a large number of

horses are to be tested, this method has a great advantage over the subcutaneous one on account of the short time expended in making the test. With practice and sufficient assistance to control the animals, as many as several hundred horses can be injected within an hour. The reaction manifests itself by local symptoms, such as swelling of the eyelid, conjunctivitis, lacrymation, and later a muco-purulent discharge, which freely flows from the eye. The reaction is typical at about the twelfth to twenty-fourth hour after injection. This test is now considered to be thoroughly reliable, but in a small number of cases doubtful reactions may be met with; in these cases the animal should be subjected to a subcutaneous test later. In non-reactors there may be a slight swelling of the lower lid, but this is easily distinguished from the intense inflammation and muco-purulent discharge so typical of a positive reaction. Should there be any suspicion that a seemingly positive reaction was caused by some other agent than the mallein employed, a second test should be made on the other eye twenty-four hours later.

Actinomycosis.—Actinomycosis is an infectious disease of cattle, occasionally affecting other domestic animals and man, which manifests itself either in the appearance of connective-tissue tumours or in chronic processes of suppuration.

Etiology.—The ray fungus, *Streptothrix actinomyces*, and *B. actinomyces*. This is a very common disease

among cattle, and until quite lately its treatment has been confined to the use of iodine, given in the form of some of its salts. Iodine was considered a specific for this disease, but personally I consider it not to be, and my view is supported by several practitioners.

Lately, in human practice, autogenous vaccine has been used in the treatment of this disease with remarkable success. In the spring of 1920 I employed an autogenous vaccine for the treatment of a large actinomycotic tumour in the parotid region of a cow. In this case potassium iodine had been used for months without any success. I treated this case with an autogenous vaccine for two months, giving injections of increasing doses every ten days; by the end of the treatment the tumour had practically disappeared, the sinuses which had been discharging pus were completely closed, and the animal had regained her normal healthy appearance. Unfortunately, I lost sight of this animal, so cannot say if the cure remained permanent or not. In the *Record* of June 18, 1921, Mr. W. M. Scott, F.R.C.V.S., records a case of this disease treated with vaccine; the cure was complete. In this case Mr. Scott used in all thirty injections of vaccine at intervals of seven days before the cure was complete, but as this animal appeared to be a very bad case with, apparently, internal complications, it may account for the necessity of the extended treatment. He, however, records that the cure was permanent, as, after a lapse of two years, the cow remained healthy, without any sign of the disease returning.

However, in treating animals with actinomycotic tumour of the glandular tissues, one must be careful to ascertain that the lesion is not tuberculous, with, perhaps, actinomycosis as a secondary infection. In cases of this kind, which, of course, would not respond to vaccine treatment, doubt might be cast upon the efficacy of the vaccine. In preparing an autogenous vaccine in cases of this kind, it is advisable to examine the material taken from the abscess or tumour for the presence of acid-fast bacteria.

Recently considerable doubt has arisen as to the ray fungus being entirely responsible for the lesions of actinomycosis. Mr. L. Colebrook, M.B., B.S., has shown that, at least in the human subject, lesions simulating those of actinomycosis are often caused by a bacterium which is called the "actino-bacillus." Lignières and Spitz have described an infection in cattle resembling that of actinomycosis, the causative agent of which is a Gram-negative bacillus, the same as the actino-bacillus. To this bacillus they give the name "actino-bacille." Colebrook points out that lesions for which these latter organisms are responsible do not in any way respond to iodine treatment, but are amenable to treatment in many instances with autogenous vaccines. These facts may explain the failure of certain cases of presumed actinomycotic lesions to respond to the supposedly specific iodine.

Botryomycosis.—Botryomycosis is a chronic infectious disease of equines. It usually results in the

formation of local tumours resembling fibromata, and occasionally in the formation of metastases in the internal organs. Abscesses often form in these tumours, which burst, and a running fistula remains, or there may be several small fistulæ opening on different parts of the tumour.

Etiology.—The *Botryomyces equi*, or *Micrococcus ascoformans.*

Lesions of this disease occur frequently on the shoulders of horses, in the mammary gland and the spermatic cord after castration.

Treatment.—I have successfully treated three cases of this disease with autogenous vaccine.

1. A driving horse, with a large tumour at base of neck, which surrounded the jugular vein. On account of its involving the vein, I refrained from removing the tumour by surgical measures. Instead I opened into the pus cavity, and obtained a swab, from which an autogenous vaccine was prepared. The vaccine was employed at intervals of eight to ten days, each dose containing an increased number of cocci. The initial dose consisted of 80 million cocci, each subsequent dose increased by one-fourth the number of the preceding dose. Altogether twenty injections were made, by the end of which the tumour had disappeared; all that remained was a thickened condition of the overlying skin.

2. Large tumour in right half of mammary gland of a thoroughbred mare. This tumour contained four fistulæ, from which a thick sticky pus was flowing.

This case was treated as in No. 1. Eighteen injections were given, by which time the tumour had disappeared; the affected half of the gland, however, was, on completion of treatment, smaller than the other, and had a shrunken appearance.

3. A two-year-old colt suffering from schirrous cord on left side. Cord was removed as high up as possible in inguinal canal, without actually entering the peritoneal cavity. The operation wound did not completely close, a small fistula remaining, which continued to discharge pus. From this pus an autogenous vaccine was prepared, initial dose being 10 million organisms, subsequent doses increased as in No. 1. Altogether eight injections were given, by which time the fistula had completely closed, and the animal was again normal. So far as I can ascertain, there was no relapse in any of these three cases. I consider that vaccine treatment in cases like the above is a valuable adjunct to surgical measures, but more clinical evidence is required to establish treatment by vaccine, in these conditions, upon a firm basis.

Infectious Abortion of Bovines.—Infectious abortion is a very common disease of cattle, from which enormous economic losses are sustained by farmers and breeders throughout the world. Under the term "infectious abortion" are included those cases which occur in otherwise healthy animals, as a result of infection producing inflammatory lesions of the uterine

mucous membrane, of the fœtal membranes and the fœtus itself.

According to the work done in recent years, the term abortion, as indicating a specific distinctive disease, is misleading. Abortion is not a disease, but one of the symptoms of a variety of diseases caused by a variety of infections.

Etiology.—Since the discovery by Bang of a short bacillus, which he called the *B. abortus*, and which he claimed to be the cause of abortion, this disease has been everywhere considered to be the result of infection by the *B. abortus*. Recently, however, dissension has arisen among workers upon this disease as to the specificity of *B. abortus* in producing the lesions responsible for abortion. Notably among the workers who disclaim this specificity are such authorities as Professor Williams and numerous other well-known clinicians in America, and most Danish authorities now hold this later view. From clinical experience I am in accord with the non-specific theory of contagious abortion.

The commonest organisms which are found to be associated with lesions responsible for abortion are—

1. *B. abortus.*
2. Streptococci.
3. Colon bacilli.
4. Vibrios.
5. Spirilla.
6. *B. pyocyaneus.*

Immunisation.—The method of immunisation re-

commended by McFadyean and Stockman for the once almost universally accepted specific infection by *B. abortus* has been extensively employed in this country. The procedure of immunisation is as follows: 50 c.c. of a living virulent bouillon culture of the *B. abortus* are injected subcutaneously six to eight weeks before the animal is put to breed. After injection the animal shows depression, loss of appetite, and a decrease in milk supply; there is also swelling and tenderness over the seat of injection. These symptoms continue for about twenty-four hours, when the animal may regain the normal, or the tenderness at the seat of injection may continue and an abscess form; this latter sequel is quite common. Immunisation by the above method is now falling into disuse in many countries where it had been extensively employed, for the reason that it is now considered a great means of continuing the infection, and disseminating it to healthy animals. Infectious abortion of mares is caused by a streptococcus, the *B. abortus equinus*. No work is recorded relative to immunisation to this disease.

Infectious Vaginal Catarrh of Cows, or Granular Venereal Disease.—Infectious vaginitis is a chronic infectious disease of cattle which is principally characterised by peculiar nodule formations on the vaginal and vulvar mucous membrane.

Etiology.—A Gram-negative streptococcus is considered to be the cause. This disease is found very frequently among cows; infection is spread very

commonly by the bull, but may also be carried by litter contaminated with the discharge from the external genitals of an infected animal.

Treatment.—When the disease is confined to the vulva and vagina, it yields to local medicinal treatment; but it commonly spreads to the cervix, uterus, Fallopian tubes, and even the ovaries, establishing a chronic inflammation in these organs. Under these circumstances it becomes a serious affection, especially when the Fallopian tubes and ovaries are involved, as sterility is always the sequel. In the above conditions an autogenous vaccine should be prepared, the causal organism being procured by taking a swab of the uterine discharge.

In chronic salpingitis, which is very often associated with cystic ovaries, very favourable results follow treatment with an autogenous vaccine. As the cystic condition of ovaries is secondary to the endometritis or salpingitis, and often intimately associated with these conditions, disappearance of the ovarian cysts follows the disappearance of the primary lesions in the uterus or Fallopian tubes. In the many cases of nymphomania seen in mares and cows, the primary cause is either an endometritis, followed by salpingitis, or either condition may act separately as an excitant to the production of ovarian cysts, which give rise to the condition of nymphomania. By treatment of the primary lesions and effecting their cure, in the great majority of cases the ovarian cysts will disappear, and likewise the objective symptoms

of nymphomania. I will give some examples occurring in my own practice.

1. A shorthorn pedigree cow became infected with granular vaginitis, presumably from a bull. This animal was treated for the vaginitis with astringent antiseptic douches. After about ten days of this treatment the vaginitis had presumably disappeared. A month later the animal was put to bull, but did not become pregnant. After several subsequent services the animal remained sterile. The owner was very anxious that this animal should breed, so a thorough examination of the generative organs was made. Vaginal examination revealed an inflammatory condition of the external os uteri, with a muco-purulent discharge from the cervix. Rectal examination revealed a thickening of the right Fallopian tube to about the size of a lead pencil. The right horn of the uterus was much larger than the left. Diagnosis: Cervicitis, endometritis, and salpingitis.

Treatment consisting of intra-uterine douches of Lugol's solution was commenced. After about three weeks of this treatment the inflammatory condition of the os uteri and thickening of the right horn had disappeared; however, enlargement of the tube remained, together with a slight uterine discharge, or, to be more precise, the uterus contained a small quantity of muco-purulent fluid. A swab of this discharge was taken and examined microscopically; streptococci were found. From these streptococci a vaccine was prepared, and on the twenty-fourth day of treat-

ment vaccination was commenced. Increasing doses were given at intervals of seven days; in all ten doses were given. The intra-uterine douches were continued once a week. By the time the tenth dose of vaccine was given the Fallopian tube had apparently returned to normal, and the intra-uterine contents was normal. Three weeks later the cow was put to bull and proved in calf.

2. A hunter mare had a difficult foaling, followed by a slight metritis. This, however, became chronic, and an endometritis persisted. That season the mare did not prove in foal. I examined her in August and found the uterus subinvoluted; there was a slight discharge from the os. Dilatation of the os revealed cervicitis and endometritis.

Medicinal treatment as in No. 1 was employed, but as the uterine discharge persisted at the end of a fortnight's treatment the discharge was examined; streptococci and bacilli of the colon type were found. A vaccine was made from the streptococci present. In all eight injections were given at intervals of seven days, the uterine douches being also continued. After the eighth injection of vaccine the discharge from the uterus had ceased. A swab of uterine secretion was examined and found sterile. From that date treatment was discontinued. Next season the mare was sent to stud and bred a healthy foal.

In several other cases of affections of the generative organs I have used vaccines with much success, and consider them a valuable adjunct to medicinal treat-

ment. Where the bull becomes infected from cows suffering from a pyogenic vaginitis, a urethritis often develops; this often resists the most rigid medicinal treatment, as the infection continues to persist in the urethra at the curvature of the penis. In this condition vaccines should be employed, together with medicinal treatment in the form of urinary antiseptics and the local application of astringent antiseptic solutions.

CHAPTER VIII

BACTERIAL DISEASES OF THE CENTRAL NERVOUS SYSTEM

Epizootic Cerebro-Spinal Meningitis (Borna Disease, Cramp of the Neck).—Epizootic cerebro-spinal meningitis of the domesticated animals is an independent infectious disease, characterised by inflammation of the membranes of the brain, spinal cord, and adjacent nerve tissues. In certain districts the disease is enzootic, and may even tend to be epizootic. In many cases there are no macroscopic lesions.

Etiology.—The disease is due to an infection. The organism commonly found is a streptococcus, either in the form of micrococcus or more rarely a diplococcus. Sometimes it may be caused by the tubercle bacillus.

This disease is quite common, chiefly in the enzootic form, in young animals. I have repeatedly seen it in young calves, and on three occasions in yearling colts, occurring a month or more after castration. On the Continent and in America this disease occurs frequently in the epizootic form, when the death-rate is very high. Recovery from one

attack does not confer immunity; in this way it resembles nearly all cocci infections.

Treatment.—As many authors contend that the causative organisms in this disease are the same or very closely related to the *Diplococcus intracellularis meningitidis*, which causes the same disease in man, the use of the serum prepared for treatment of this disease in humans is indicated. In man the best results have been obtained from the use of a polyvalent immune serum, injected intrathecally (for description of this procedure see Tetanus). Serum used in the human subject in this manner reduced the mortality by half. Personally, I have treated one case of cerebro-spinal meningitis with human anti-meningococci immune serum. The subject was a month-old Aberdeen Angus pedigree calf. The injections were made by lumbar puncture. Three injections of 30 c.c. serum at intervals of forty-eight hours were given, recovery following. The only trace of the disease which remained was a slight difficulty in regaining the standing position when the animal lay down; he would get on to his knees and remain in this position for a minute or so before finally getting on his feet.

In valuable animals suffering from this disease serum treatment should be employed, the serum prepared for human meningitis being easily obtainable. If this cannot be procured, ordinary normal serum should be used, preferably obtained from an animal of the same species as the one on which it is

to be used, as a homologous serum is often more effective.

Enzootic Spinal Paralysis of Equines.—This is, as a rule, an acute infectious disease of equines, characterised by multiple capillary hemorrhages in various organs, and especially in the spinal cord. The special clinical feature is paralysis of the posterior parts.

Etiology.—A specific streptococcus, the *Streptococcus melanogenes* of Schlegel.

This disease is very common, occurring in an enzootic form, and very often attacking in-foal mares in poor condition. In the acute form the mortality from this disease is as high as 98 per cent.

Treatment.—So far a specific antiserum has not been prepared from the causal organism, but if such a serum was obtainable it should give good results, if given intrathecally, in the treatment of this disease. In the absence of this specific serum normal horse serum should be employed. This should be given intrathecally, after first withdrawing the cerebro-spinal fluid by lumbar puncture. The *modus operandi* here is the same as that described under Tetanus.

Various Chronic Affections due to Pyogenic Bacteria.—There are numerous conditions existing in a chronic form which yield to vaccine treatment. In all these cases best results are obtained from the use of autogenous vaccines, in conjunction with local

surgical treatment of the lesions. Cases of this kind are chronic suppurating sinuses—as poll evil, fistulous withers, pyorrhœa alveolaris, or suppurating periodontitis. In this latter disease there are generally numerous organisms present, and a polyvalent vaccine should therefore be employed. Chronic inflammation of the eye, such as conjunctivitis caused by pyogenic organisms, yields readily to vaccine treatment or antistreptococci serum.

Preparation of Vaccines.—For all practical purposes vaccines may be regarded as suspensions containing intracellular toxins in combination with bacterial protoplasm, and the results obtained by their employment depend upon the activity of these toxins and the quantity present in the vaccine. Such vaccines may contain (*a*) dead bacteria, now commonly called "bacterins"—the bacteria are destroyed either by heat or chemical reagents; (*b*) bacteria in the "living" condition, now spoken as "true vaccine," either unaltered, attenuated, or "sensitised" by combination with the homologous immune body. A vaccine may be "autogenous"—that is, prepared from the particular strain of bacterium already producing the infection in the patient; or "stock"—that is, one prepared from another bacterium of the same species, but already stored in the laboratory. However, a few general principles may at once be laid down with regard to their preparation. In the first place there is a consensus of opinion that as the passage of an organism through the body of each individual

has modified to a greater or less extent its biological characters (resulting in the establishment of a number of "strains" of that organism), the best results will be obtained by utilising an autogenous rather than a stock vaccine.

In some cases the preparation of an autogenous vaccine is a matter of great difficulty, if not of impossibility, owing to the difficulty of isolating the responsible organism; but it should always be attempted, although a stock vaccine may be utilised during the interval.

The organism must be as virulent as possible, and to retain this character the subcultivations used in the preparation of the vaccine must not be far removed from the body of the patient. In other words, the isolation of the bacterium from the morbid material must be effected as rapidly as possible and in as few generations as is consistent with obtaining it in a state of purity. Next, the particular subcultivation intended for the production of the vaccine must be cultivated under "optimum" conditions.

The actual process of preparing a vaccine is briefly as follows: The organism responsible for some given infection, having been isolated from the lesion existing in the patient and identified, is planted upon a suitable medium, and is incubated under optimum conditions for such period of time as experience shows is calculated to yield the maximum number of living vigorous bacteria. At the end of the cultivation period the growth is examined visually to determine

its freedom from gross contamination, and by means of stained preparations to determine its purity. The culture proving satisfactory, 5 c.c. of a 0·1 per cent. saline solution are pipetted into the tube or bottle, and the growth emulsified as evenly as possible with the help of a glass rod. The turbid emulsion is transferred to a stout test-tube, containing a number of glass beads; this is then placed in some form of a mechanical shaker, and agitated thoroughly for about fifteen minutes.

Standardisation of Vaccines.—The amount of bacterial protoplasm present in every cubic centimetre of the emulsion is next estimated by weighing or by Wright's method. This latter method is now the one perhaps in most common use. It consists in taking equal volumes of blood from a normal individual and of the bacterial emulsion, mixing thoroughly, spreading in a thin film on a glass slide, fixing, and staining with Leishman's stain, and then with the help of a $\frac{1}{12}$ oil-immersion lens enumerating the number of red cells and bacteria respectively in some twenty-five separate "fields" of the microscope. From the number thus recorded an average is struck, and the ratio the red blood bears to the bacteria is estimated. Assuming that normal blood contains 5,000 millions of red cells per cubic centimetre, a simple sum in proportion gives the number of bacteria present in each cubic centimetre of the bacterial emulsion. By the addition of 0·1 saline solution to each cubic centimetre of the

bacterial emulsion, it can be reduced to the desired number of bacteria in each cubic centimetre. The mixture is then well shaken. The tube is then suspended for a period of thirty minutes in a water-bath running at a temperature corresponding with the thermal death-point of the bacteria for the vaccine. After removal from the water-bath loopfuls of the emulsion are sown upon suitable media and incubated, in order to determine the sterility of the vaccine. Finally, a small quantity of antiseptic is added to the vaccine, which is then put up for use, either in glass capsules or bottles or tubes closed with a rubber cap.

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